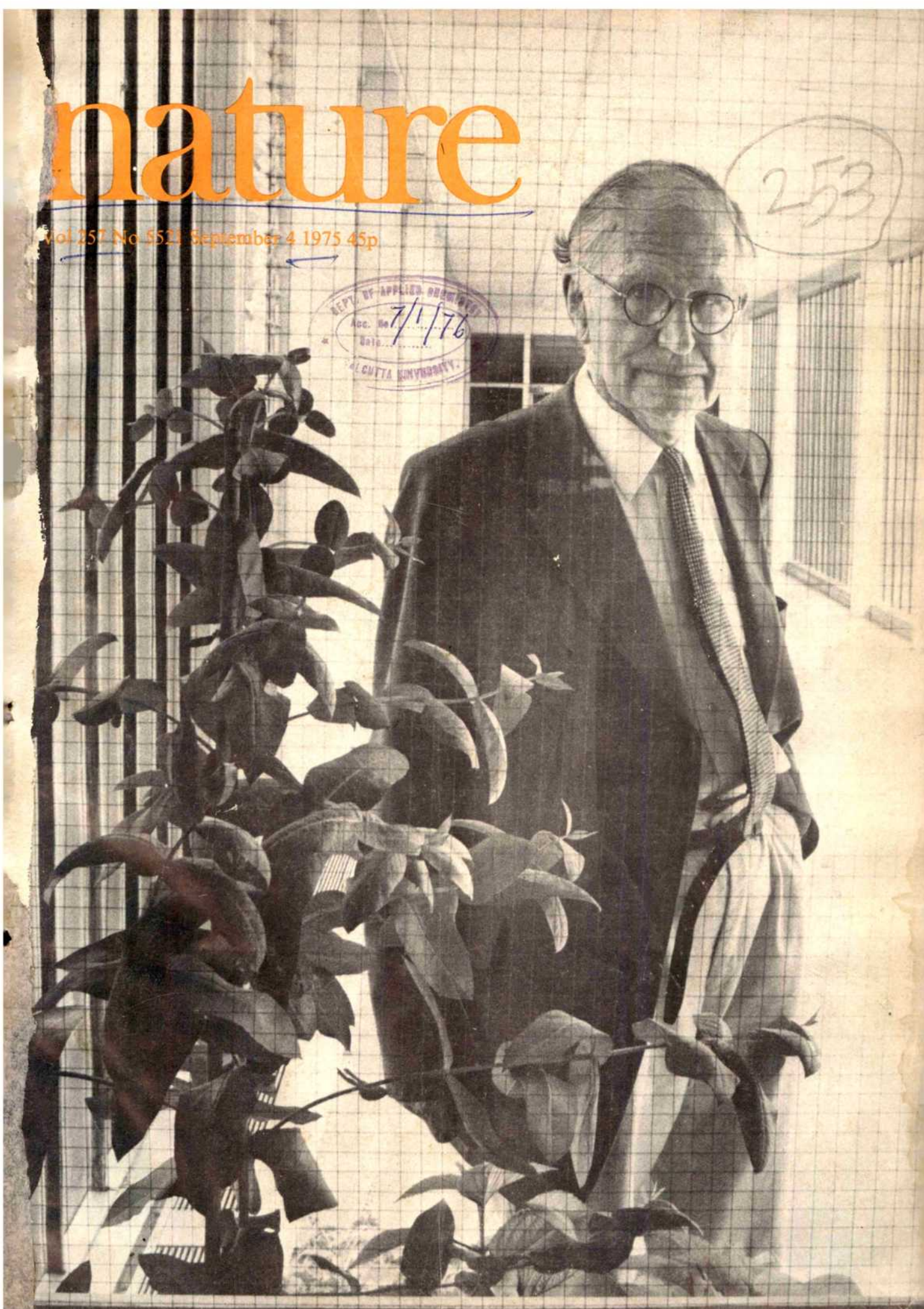


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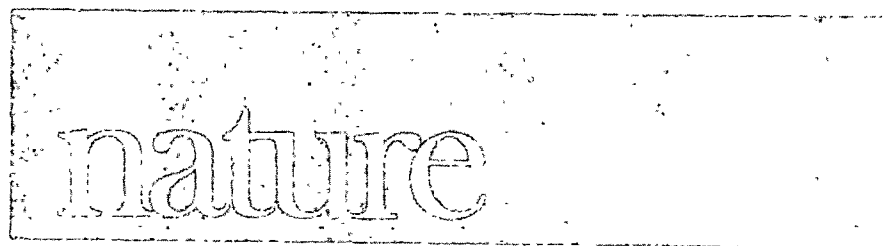
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flame to the chamber is modified by
an electric field.

[S. S. Sandhu, Imperial College]

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Singular Optimal Control Problems

David J. Bell and David H. Jacobson

October/November 1975, xii+190 pp., £5.80/\$15.00 0.12.378750.5

The purpose of this book is to collect together all known results in optimal control theory (as well as appropriate computational methods) which can be applied to the singular problems in optimal control and which up to now have been scattered in numerous journals. Complete and self-contained, the volume begins with an historical survey of singular control problems and leads to the presentation of important, recent results in the field. There are specific real-world applications and the authors discuss those avenues of research which require further investigation.

Studies in Archaeological Science

Soil Science and Archaeology

Susan Limbrey

October/November 1975, xvi+342 pp., £8.40/\$21.75 0.12.785477.0

This book, both a work of reference and a field manual, clarifies the relationship between people whose remains are the subject of archaeological study and the soils upon which those people depended for food and resources. Dr. Limbrey gives an account of soil processes, which have an important effect on the distribution and condition of material remains, the formation of soil profiles and the consistency of soils. She then considers the distribution of soil types in terms of the environment under which they have formed and the history of their development under changing conditions. Those British soils strongly influenced by man's activities in disturbing, clearing and cultivating the land are described in detail, as are soils of other regions. The final section deals with soils in relation to the work of field archaeologists.

Computer Science and Applied Mathematics: A series of mono graphs and textbooks

Graph Theory An Algorithmic Approach

Nicos Christofides

October/November 1975, xvi+400 pp., £12.50/\$32.25 0.12.174350.0

This book deals with the algorithmic and computational aspects of graph theory, to the virtual exclusion of pure theory. The author's primary aim is to acquaint his audience with the main techniques for the solution of important graph theory problems, to develop new algorithms and to unify and relate these methods into a coherent body of knowledge. Intended for those working in operations research, computer science and civil, transport and electrical engineering, the book will leave the reader in a position to relate and adapt the basic concepts to their own fields of application, and derive new ways of solving their own problems.

Leucocytes: Separation, Collection and Transfusion

Proceedings of the International Symposium on Leucocyte Separation and Transfusion held at the Royal Postgraduate Medical School, London in 1974

edited by J. H. Goldman and R. M. Lowenthal

October/November 1975, xxxvi+602 pp., £13.80/\$35.75 0.12.288550.3

The separation and collection of leucocytes from appropriate donors and their transfusion to patients with malignant disease or aplastic anaemia is a rapidly developing aspect of supportive care. This book contains the proceedings of an International Symposium convened in recognition of the recent advances in the technology required both to separate leucocytes from the peripheral blood and use collected granulocytes in the treatment of infection in leucopenic patients. Other uses of continuous blood flow blood cell separators were also considered: these include collection of acute leukaemia cells, treatment of chronic leukaemia and plasmapheresis.

The Role of Fats

edited by A. J. Vergroesen

October/November 1975, xiv+494 pp., £14.00/\$36.25 0.12.718050.8

Dr. Vergroesen and his colleagues discuss the pathological effects of fats in relation to diabetes, atherosclerosis, arterial thrombosis and obesity, analysing the process of fat absorption and the role of different fatty acids. They consider the possibilities of preventing any harmful effects produced by certain fats, particularly rapeseed oil which is used extensively for domestic consumption. The book includes a lengthy discussion on the importance of vitamin E in its capacity as a natural antioxidant. It is claimed that an increase in consumption of polyunsaturated fatty acids will lower the tendency of blood platelets to adhesion and aggregation, so decreasing the risk of arterial thrombus formation.

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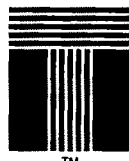
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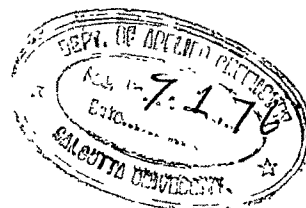
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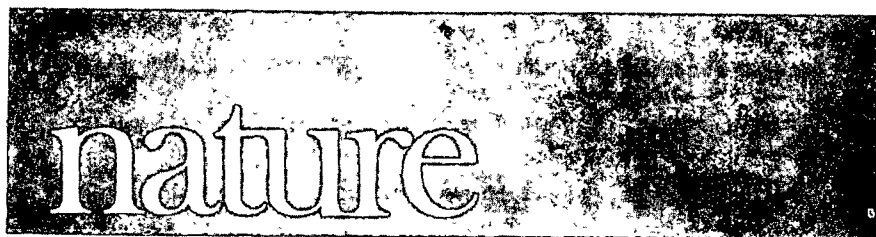
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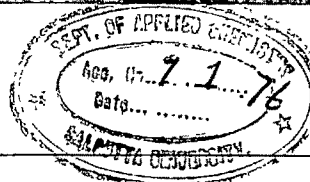
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How much planning is enough?

REPORTS by the OECD on national policies for scientific research and the like have never been exactly bed-time reading for most scientists. Essential raw material, certainly, for politicians, civil servants and those who study the policies that emerge, but until recently little more than titles on a bookshelf to others. All that is changing, however, as the man on the laboratory floor increasingly realises that his own future is so inextricably bound up with the policies made in London, Paris or Washington that he simply has to take an interest.

The emergence of another weighty OECD report is therefore, perhaps, the time for a few more people to become acquainted with the kind of data on science that that organisation provides. This particular report is called *Changing Priorities for Government R & D* and, be warned, you need plenty of time (and a magnifying glass) to digest it all properly; even the authors admit that "this is by no means a short report".

One of the conclusions reached in the report, and perhaps not a very startling one, is that none of the 12 OECD countries studied had anything like a foolproof system whereby the allocation of funds and their breakdown between activities could be planned in advance through an overall 'science budget' which reflected priorities laid down as part of a government's overall policy. That may sound a laudable thing to be aiming for, until one comes to realise that some 10% of the total government expenditure on research and development in France and the UK goes to the "advancement of science via general university funds" (through the University Grants Committee in the UK), that in countries like the Netherlands, Norway and Sweden the figure is between 20% and 45%, and that in Japan it is a staggering 60+. Persuading universities successfully that such and such a list of priorities is the right one is tantamount, at least in the UK, to removing much of their autonomy, any talk of which is liable to make the new and diligent bed-time reader of OECD reports toss this latest document to one side.

This brings to mind the calls made a year or so ago by the UK Parliamentary Select Committee on Science and Technology for a Minister of Research and Development to be appointed, and the general opprobrium that was heaped on the idea. Few would support the regimentation in such a way of an area for which regimentation is so obviously inappropriate.

Leaving aside the question of whether completely planned science is necessarily good and useful science, as the OECD seems tacitly to assume, the OECD report contains some interesting revelations about the way the 12 selected countries allocated and divided up their funds for research and development. The policy-making

machinery in each country, although running true to a basic pattern, is nonetheless definitely unique, and yet most of the 12 have come to allocate much the same fraction of total government expenditure to research and development, a trend which became particularly marked in the early 1970s. In 1961, for example, the USA, the UK and Germany spent, respectively, 10.5, 8.5 and 3.7% of their total expenditure on research and development, but by 1971 these figures had become 7.5, 7.1 and 6.0%. Coincidence? Or have three quite different systems for deciding the funding of research and development come up, roughly, with the 'right' answer even in the absence of complete planning and the complete ordering of priorities.

A closer examination of the priority accorded to research and development activities such as 'civil nuclear', 'civil space' and 'defence' reveals more similarities, but also some discrepancies. For example, the authors of the OECD report have set up, for each country, a league table which places expenditures on each of 14 research and development areas in order. Comparing these league tables for the 12 countries shows that the UK, Germany, France and Italy have roughly the same ideas about how to divide up research and development funds, and that Norway, the Netherlands, Belgium and Sweden also work to their own characteristically similar lists of priorities. On a more detailed level still, however, there are of course differences even between countries with superficially similar scientific aims; France and Italy, for instance, spend 1.6 and 0.4% respectively, of total government expenditure on 'civil nuclear'. Not surprisingly, the greatest disparities are to be found in the realms of defence research, with the USA, the UK and the Netherlands, for example, spending 4.9, 2.9 and 0.3% of their total expenditure on it.

Although items such as these appear near the beginning of any government's shopping list for research, the other end of the list contains some entries which are all too often glossed over or ignored in discussing a country's research programme. One such is research and development for the benefit of developing countries. The situation is epitomised by the fact that for 5 of the 12 OECD countries surveyed, no data were available, indicating either that a negligible amount was spent or that the amounts were hard to pick out from the general research and development background (and therefore hard to plan). France and the USA spend most, some \$20 million each in 1972, which represents 1.5 and 0.2% respectively, of the total government funding for research and development—not really too impressive when the target set for the UN Second Development Decade is 5%. □

A new way to slay old pests

Tests are under way at the Microbial Research Establishment, Porton Down, to determine the safety of two viruses which may be used in the control of insect pests. News of the work, which is being carried out under contract for the Ministry of Overseas Development, was given by Dr T. W. Tinsley to members of the British Association, gathered for their annual meeting at Guildford last week. Dr Tinsley, who is Director of the Natural Environment Research Council's Unit of Invertebrate Virology at Oxford, described the potential of viral control to Peter Collins.

AWARENESS of the potential of the virus control of an insect pest dates from the 1930s. At that time, the European spruce sawfly, *Gilpinia hercyniae*, which was causing catastrophic damage to spruce forests in Canada, began to decline in numbers and within a few years was no longer an economically important pest. The reason was found to be infection by a virus, which in Europe was one of the factors controlling the natural sawfly population. But development of the possibilities opened up by this fortunate incident was delayed by the Second World War and then inhibited by the discovery of the vast range of new chemical pesticides which set the pattern for pest control in the immediate post-war era. This pattern was to last at least as long as the commercial profitability of the chemical pesticides, especially when it was found that the research required to develop viruses for this purpose was possibly as costly, and certainly as time-consuming, as the development of new chemical agents.

There are several reasons why the situation has changed in recent years. Initially, increasing resistance on the part of pests of agricultural as well as medical importance drew attention to the advantage of all types of biological control. Further pressure in the same direction resulted from increased concern at the environmental effects of many "classical" pesticides. More recently, two even more potent factors have given impetus to all research in this field: first, the growing serious-

ness of the world food situation, and hence the urgent need for more efficient control of all manner of pests and diseases, and, second, the enormous and sudden rise in the cost of all chemical products.

The part that may be played by viral agents in this new era of pest control, and the reasons why the advance into this field must be gradual and cautious were clearly stated by Dr Tinsley in his contribution to the symposium on the work of the research councils at Guildford last week. As Director of NERC's Unit of Invertebrate Virology (UIV) at Oxford, Dr Tinsley indicated what has been achieved so far and gave some idea of what may be expected in the next few years, at least within the unit's terms of reference.

For reasons of safety, and in particular because of the risk of cross-infection with higher animals, the WHO and the FAO have recommended that among the seven groups of viruses known to occur in insects, only the baculoviruses should be considered as pesticidal agents. So far as is known, these have neither chemical, physical nor biological properties in common with any virus found in vertebrates or plants, thus limiting the risk of cross infection to other insects or, at worst, invertebrates. Within this group, the unit's work is at present concentrated on the nuclear polyhedrosis viruses (NPVs) which attack four members of the Lepidopteran genus *Spodoptera*. The larvae of these Noctuid moths are now among the world's most serious pests, attacking a wide range of important crop plants from grass to fruit trees in Asia, Africa and across the Atlantic. In particular, interest has been concentrated on *S. exempta*, the East African army worm, possibly the world's most serious grass-land pest. Able to produce up to 11 generations in a single year, it is found from southern Tanzania to Ethiopia, and its outbreaks have been known to denude up to 300 square miles of pasture in a few weeks. Not surprisingly, this work is being actively supported by the Ministry of Overseas Development (MOD), and the unit works in close collaboration with MOD's Centre for Overseas Pest Research.

One of the major problems in the use of viral pesticides hitherto has been the lack of precise information about the nature and characteristics of the viruses being used, and it is towards filling this gap that the unit's programme is directed. Its prime objectives were defined by Dr Tinsley as "the investigation of the chemical, physical and biological properties of insect viruses, and then determination of the effects on the insect hosts and the manner in which the viruses spread under natural conditions". The first of

these objectives involves fundamental research in a field where little such work has been done, and in fact the unit is here leading the world.

To be of value in developing any virus as a biological control agent, four distinct steps have to be taken, and these too have been precisely defined. They involve:

- The isolation, purification and detailed characterisation of each virus in such a way as to enable its unequivocal identification. (It is surprising that this has not even been done previously for the viruses which are already in use as pest control agents in various parts of the world.)

- Testing the purified virus under laboratory conditions to assess its efficacy and establish its host range.

- Testing the toxicological properties of the virus "together with any associated formulative materials". This must be accompanied by investigation of the possibility of infection of, and replication in, non-target invertebrates and vertebrates.

- Field trials, on an increasing scale, on the crop and in the areas in which it is intended to use the virus as a pesticide. To do this final step satisfactorily, some system of monitoring needs to be evolved, and such monitoring will have to be continued for several years. It is thus apparent that the whole process is a lengthy one, and any idea that viral pesticides can provide an immediate panacea, even within the range of pests against which their use is intended, has to be forgotten.

Only the first two steps are the responsibility of the UIV at Oxford, and already some of the results are apparent. Thus, examination of the viruses of the four species of *Spodoptera* has indicated that these viruses, although each distinct and recognisable, fall into two groups or serotypes, each with several closely related strains. This has gone some way towards answering the question whether or not, as has hitherto been taken for granted, insect viruses are specific—a matter of the utmost importance if any such virus is to be widely distributed in a natural environment. Moreover, again to quote Dr Tinsley, "this is the first time that such a detailed investigation has been undertaken and also the first time it has been possible to make unequivocal identifications of any of the baculoviruses". Moreover, until this had been done, "it was not possible to be certain that the host insects had died from the test virus, from a second unrelated virus which occurred as a latent infection, or from a cross-contamination"—points that have needed to be ascertained before the second step could be undertaken with any confidence.

When sufficient purified and identified material of any of the viruses is available, it passes to the Microbiological Research Establishment (MRE) at Porton, working under contract from the MOD. One of the problems that is arising at this third stage is that although the MRE has the necessary facilities for undertaking any tests that may be required to check the toxicity of the viruses for vertebrates, their staff have no experience in handling, or working with, insects—and any results from Porton will need to be checked against the insect host. Arrangements are therefore being made, with the cooperation also of the Medical Research Council, for the closest possible collaboration between MRE and UIV at this stage. Only when a virus has been cleared of any potential ecological hazard at the laboratory level can the fourth stage of field testing, in the areas where its use is envisaged, be started. The first candidate to reach this stage is likely to be the virus affecting *Spodoptera exempta*, if only because of the MOD's very special interest in protecting the grasslands of the Commonwealth countries (and others) of East Africa. Meanwhile, however, research into the ecology of an active, natural outbreak of an insect virus is also being carried out by staff from the UIV. The insect concerned is, again, the spruce sawfly, *Gilpinia hercyniae* an outbreak of which was identified some years ago in the Forestry Commission's Hafren Forest in Montgomeryshire, UK. Dr Tinsley was already applying for permission to experiment with the relevant virus at Hafren, when, in 1970, a natural infection was discovered in the forest, which thus became an ideal field laboratory for research into the ecology and epidemiology of a natural insect virus outbreak.

Although in the UK this fundamental approach to the development of viral pesticides has been adopted and is being rigorously pursued, the same is not true elsewhere. Viral pesticides have for some time now been on the market in the USA and they are known to have been used extensively for some years in the USSR, particularly against pests of forest trees and certain industrial crops. What is happening in Russia is not at all clear, nor is it known what precautions are taken or what problems, if any, have arisen. But certain of the difficulties that the pesticide industry may face, when it comes to commercial production of a virus that has successfully passed through all four preliminary stages, have recently become evident from American experience. The viruses concerned are again those affecting certain lepidopterous larvae, namely the genus *Heliothis*, including

H. zea (on cotton, and probably the UIV's next target insect), *H. virescens* and *H. armigera*, pests of tobacco and maize respectively; the area on which *Heliothis* NPV has been used is said to be as much as five million acres. Successes in field trials on a vast scale led, in 1971, to a "temporary exemption from tolerance requirements" on the part of the ES Environment Protection Agency, and when this was later confirmed, the way was clear to go ahead with commercial production. Two companies, International Mineral Corporation and Nutrilite, put viral pesticides on the market—Viron and VHZ respectively—confident that they had at last achieved a breakthrough. Prolonged testing and field use had indicated that the degree of control was at least as good as that with chemical insecticides, whereas after 12 years of experimentation there was said to be no change in the specificity of the virus and no sign of any resistance on the part of the host. And yet, within recent months, the product actually put on sale has seemed to be less effective than those years of testing had led the manufacturers to believe. The extent to which this is so, why it should be, and how it can have happened, is not clear.

While these and other developments are going ahead in the UK and the USA, no one, in the UK at least, believes that rapid and sensationally successful control of the target pests is just around the corner. Problems will certainly arise at higher altitudes for example, unless some way can be found of getting over the undeniable fact that NPVs are inactivated by ultraviolet light (although one recent report indicated the reverse effect for the virus of one Lepidopteran, *Autographa californica* at least when grown *in vitro*). Then there seem to be problems relating to the effect on the virus of various leaf surfaces. A great deal of work will have to be done on the evolution of suitable formulations for large scale manufacture and application of the commercial product—especially in view of recent American experience. Finally, there is some uneasiness about the adequacy of the protocols for safety testing, so far developed by the WHO on the basis of those used at present in the United States—and themselves developed for non-replicating, easily identifiable chemical pesticides.

But once these new pesticidal agents become available, unequivocally identified and rigorously tested, in standardised formulations that can be used in complete safety, a new and most powerful weapon will be available against many of the pests that threaten the world's essential food supplies and industrial crops □

KENNETH MELLANBY



Mathematics out of molehills

THERE is generally an optimum period for any worker to continue in a particular field of research. It takes a little time to become familiar with a new topic, productivity may then build up, but eventually he becomes stale and the "law of diminishing returns" seems to ensure that progress becomes slower and slower. This is the time to change direction, though after a fallow period the field may once again become productive.

These musings were prompted by a reader who writes: "Last week I was staying on a farm and looked at mole tracks as the moles went about ruining a newly seeded lawn. Why do the tracks so often run at right angles; they seem to turn through 90° at various points along their track. Perhaps they are particularly geometric beasts". Now I have been interested in moles for years and have even produced a monograph on the subject, but familiarity has evidently prevented me from recognising the interest of the tunnel pattern. My immediate reaction on receiving the letter was to go out and see what wild moles were actually up to. I looked with new interest at the working in my own garden, four acres of near-wilderness where wildlife is encouraged. I also returned to my main study area, Monks Wood National Nature Reserve. I may perhaps remind readers that moles, like most of our native fauna, are primarily woodland creatures, though they have been able to adapt to man-made habitats like farmland and lawns. I found everywhere, and clearly recorded in my own data made in previous years in farmland, that they do indeed have a predilection for right angled bends in their tunnels, particularly in new excavations in cultivated soil. In the wood, where they live in permanent tunnels which house generation after generation of solitary moles, this pattern is not so noticeable, possibly because of modifications over the years where repairs have been executed.

Another observation revealed something which may also be a function of

I write to you from a Soviet camp for political prisoners, located in the Lesnoy Settlement in Mordovia (Camp ZhKh-385/19). The problem to which I would like to draw your attention is of vital interest not only to me personally, but also to a large number of Soviet political prisoners—scientists who are in camps for expressing their political views openly.

In this letter I will not concern myself with whether and up to what extent the persecution of citizens for exercising their right to free speech, guaranteed to them by law, is lawful and justified, though this fact in itself should also come to the attention of world scientific opinion. However, this question is too vast for a thorough examination in a letter. Furthermore, it is up to each individual scientist to decide whether he wishes to express himself on this subject or not.

I would only like to draw your attention to the conditions in which these political prisoners are held and how this affects their professional skills. Among us there are physicists, mathematicians, biologists, civil engineers, philologists, philosophers and many others. Personally, I am an astronomer, specialised in the field of meteoric astronomy and astro-biology. Each one of us, regardless of his political views, remains, above all, a scientist and aims to preserve his professional skills under any circumstances.

However, this is impossible under the conditions which prevail in the camp. A specialist who is sent to a camp is deprived of the opportunity to follow developments in his field effectively. In accordance with Soviet law, to receive literature, periodically or in any other way, published abroad is categorically forbidden. This ban extends to

literature of non-political content, including specialised literature and literature published in countries on friendly terms with the USSR. Subject to so many restrictions and in practice totally isolated from any source of information, a scientist soon feels he lags behind contemporary science and that he is losing his professional skills. In accordance with existing forms of evaluation, total disqualification is reached in approximately three years.

Letter from the Soviet Union

We are serving sentences of five, seven years and longer. We face total creative impotence. We are not just deprived of freedom for a given period of time. We are deprived of our chosen work and our profession forever.

These restrictions are imposed not only on publications from abroad. Literature published in the USSR can be received only through specialised shops, which deal by post. According to the rules operating the book market in the USSR, in practical terms this means that one can acquire literature which has been published only recently and even then only if it is in small demand and cannot be sold over the counter. Any monograph or reference book indispensable to work which has been published a few years back is impossible to acquire. To receive literature from private individuals (even from relatives) is strictly forbidden. Fear of the written word reaches such proportions that even cuttings from the Soviet press are not allowed in the mail. To receive scientific infor-

mation in private letters from colleagues—such a fashionable method during the Renaissance and widely used at present—is also impossible for us. Probably as a result of inadequate education of those who guard us during our imprisonment, letters of such 'strange' content arouse special suspicion. Their censoring takes many long months and quite often they are not even delivered. Letters from abroad excite particular apprehension.

The disqualification of scientific workers is speeded along by purely physiological reasons. We are all forced to do heavy physical work, and many of us who are no longer so young are badly equipped for it. This leaves us no time or energy for intellectual pursuits. The food is highly insufficient, low in calories and in quality (lacking in protein, carbohydrates, phosphorus, vitamins). A condition of semi-starvation is normal for us. I will not dwell on this problem further as it has already been examined repeatedly. I will only add that all this contributes in bringing about a sharp decrease of intellectual potential, and a weakening of memory, and so on.

For the sake of humanity and professional solidarity, I beg you, and through you scientists throughout the world, to intercede on our behalf in this difficult situation. I appeal to you to focus your attention on the plight of your colleagues. There is no need for political action. We only ask you to secure for Soviet scientists the right of unhindered use of scientific literature, and the right to maintain scientific contacts. Please send your colleagues in prison camps books and journals and other scientific material, and in this way ease the conditions in which they are held. —K. A. Lyubarskii

this same geometrical behaviour. Monks Wood consists of blocks of woodland, divided by grassy rides of short turf. The moles spend most of their time in an anastomosing network of permanent burrows under the trees, but this system is joined at frequent intervals by mole runs which cross under the rides. These almost always run straight across the ride; they never run along it. And the burrow comes out of one woodland block and enters the other, at almost exactly ninety degrees.

How and why this happens is difficult to understand. Why does the mole never make a right-angle under the woodland ride, which may be several yards across? Under a lawn, which the ride resembles, a zig-zag would normally occur in this distance. A mole busily burrowing underground in the wood

must, somehow, recognise that it has reached the edge of the ride, and it must receive some stimulus which causes it to burrow in a particular direction towards some goal on the other side.

My correspondent raised another, more familiar, problem. He asked: "How can one decently remove moles when they are wrecking your lawn without actually putting them to death?" This is difficult, and I usually try to persuade people to learn to live with their moles. Especially in a clay soil a mole (there is usually only one at a time in the average garden) makes a series of hills from the spoil as it digs its tunnel system, but when this is complete there is little further evidence of its presence. The mole is territorial, and will keep other individuals away.

There are various repellants on the

market. If they work, they usually divert the animals to a neighbour's garden. Generally they are unsuccessful, or they only close up a section of the burrow system. The Caper Spurge, as attractive plant in its own right, is reputed to keep the garden free, but it seldom does.

If people must be rid of moles, they have to kill them. The commercial scissor trap will catch one troublesome beast, and worms baited with strychnine will wipe out a large population. But the garden, particularly if adjacent to a wood, will always be liable to re-invasion. The same will happen if the neighbours find an effective repellant! It is best to rely on the territorial instincts of your own moles, hoping that they will do little more damage and will keep out all other members of their own species. □

international news

Tbilisi: a conference with problems

from Vera Rich

THE problem of the participation of Soviet scientists in international conferences is a long-standing one, and organising secretaries have for many years had to deal with the problem of delegates who are refused visas and substitutes who have little or no knowledge of the subject concerned but who are "approved" by the Soviet government. The case of the International Conference on Artificial Intelligence at Tbilisi this week raised the problem in a particularly acute form. Since the venue of this conference was in the USSR, the difficulties over visas and "exit dossiers" could not arise for would-be participants within the Soviet Union. Nevertheless, several Jewish refusnik scientists have been prevented from attending.

Professor Aleksandr Lerner was informed on June 5 by the KGB that it regarded the invitation from the Programme Secretary of the Conference, Professor Patrick Winston, as an "American provocation". Since then Professor Lerner has been called in by the KGB and questioned for several hours in connection with his invitation to the conference.

Another would-be participant, Ovsei Gel'man, received his long-awaited visa for Israel just in time for him to be safely out of the Soviet Union before the conference began. On reaching Vienna, he disclosed the remarkable piece of information that the physicist brothers Isai and Grigorii Goldshtein, who, being residents of Tbilisi would not have to obtain any travel or residence clearance to attend the conference, still had no idea at the end of August that they had been invited. It should be noted that these tactics of exclusion originate from the KGB only; the "establishment" Soviet scientists themselves have made no objection to the participation of the refusniks.

The Tbilisi conference has been used by more than 70 academics from British, US and Canadian universities as an occasion to protest against the treatment of Soviet scientists Valentin

A NEW Soviet base is to be established in Antarctica this year to prospect for mineral resources on the continent. Manned by a research team of 50 geologists, geophysicists and cartographers the station, called Druzhnaya Amity, will be based on the Filchner Ice Shelf at the southern end of the Weddel Sea, a region that until now has received scant Soviet attention.

According to an announcement from the official Soviet news agency, Tass, exploration will extend from the Antarctic Peninsula to Queen Maud Land, crossing an area which, until territorial claims were suspended by the Antarctic Treaty in 1961, was a part of the British Antarctic Territory that had also been claimed by both Argentina and Chile.

There is little room for doubt that the prime objective of the new Soviet team will be to prospect for mineral resources, with oil, gas and copper as the main targets. The Tass announcement stated that "the south [of the region to be surveyed] is reminiscent of the ore bearing zones of Siberia, while the west is a continuation of the American mountains [Andes] famous for their deposits of non-ferrous minerals.

"The most extensive region of continental shelf in Antarctica, a potential natural storehouse of oil and gas," the statement continued, "extends across the Weddel Sea and the Filchner Ice Shelf". Significantly, Soviet interest in Antarctica has in the past been concentrated largely on the opposite, eastern side of the continent.

Although official sources in Britain deny that there is any significance in the timing of the announcement, which followed closely the eighth Consultative Meeting between the signatories to the Antarctic Treaty in Oslo, it may force a decision on the

outstanding problem of the commercial exploitation of Antarctica. The Oslo meeting, which ended on June 20, was the first at which the signatories confronted that issue face on, and they were unable to reach any final decision. It was, however, agreed that the matter would become the subject of exhaustive international investigation involving all 13 signatories, and to that end a meeting is to be held in Paris in 1976 in preparation for the 1977 Consultative Meeting in London.

The Soviet decision to press on with preparations for eventual commercial exploitation may, however, precipitate a more positive response because of the new station's location. Sovereignty over the Filchner Ice Shelf was formerly the subject of an unsettled dispute between Argentina, Britain and Chile, and though territorial claims are suspended under the treaty, awkward legal questions would emerge should the Soviet Union uncover commercially exploitable resources in the area.

The decision to establish the base does not apparently run contrary to the Oslo recommendations and it is unlikely that the Soviet government will act to contravene the terms of the existing treaty. Though a solution to the problem remains some way off, there are indications that the signatories may eventually reach a mutually acceptable solution within the treaty, particularly as they are reluctant to throw the matter open to broad international debate. It is, therefore, likely that the Soviet Union, while attempting to forestall further prevarication on an issue of growing concern, will uphold the treaty; certainly, there is no reason why under its terms they should not establish a base in what was once British Antarctic Territory.

—Allan Piper

Turchin and Leonid Plyushch. The Western group, which includes many eminent names in the field of artificial intelligence, have petitioned the Soviet Academy of Sciences, Premier Brezhnev, Procurator General R. Rudenko and a good many more prominent officials. Noting that Turchin and Plyushch have been dismissed from their jobs and harassed "for defending the freedom of their colleagues", the

signatories proclaim Turchin's dismissal as "inexcusable" and Plyushch's hospital detention and drug treatment as "horrificing".

"We want to make it clear", they add, "that scientists around the world, and in particular members of the Artificial Intelligence community, have taken a keen interest in the fate of these men, and strongly protest against the treatment they have received."

Turchin is a cybernetician and physicist responsible for designing and implementing the language REFAL, which is used for designing other languages and for proving the correctness of programs. Jointly with Andrei Sakharov he co-authored a memo on human rights and social problems in 1970, and in September 1973 he circulated an open letter protesting against the official harassment of Sakharov. This led to his expulsion from the Moscow Institute for the Automation and Construction Industry in July 1974.

Leonid Plyushch, whose case has already been reported in *Nature*, was dismissed from his job at the Cybernetics Institute of the Ukraine Academy of Science after publishing a letter of protest against the trial of Yuri Galanskov and Alexander Ginsburg. He was arrested in January 1972 on charges of anti-Soviet activity and, after examination at the Serbsky Institute of Forensic Psychiatry, was diagnosed as suffering from "creeping schizophrenia with messianic and reformist ideas." He was not allowed to attend his own trial in January 1973, when it was decided that he should be detained in a psychiatric hospital. He remains there.

● Following the prestigious success of the Soyuz-Apollo project (at least as a publicity venture in support of detente and cooperation), the Soviet Union is pressing forward with a number of other international projects. At the beginning of August, the coopera-

tion of the space mission was repeated on a smaller scale with a joint stratospheric probe, consisting of two canisters of instruments—one Soviet and one American—mounted on a single triangular frame and carried aloft by an automatic balloon system. The apparatus, launched from a site near Ryl'sk in the Kursk region, reached an altitude of 30,000 m before descending by parachute, and the whole flight, from launch to sighting by the recovery helicopter, lasted 142 minutes. The purpose of the experiment was investigation of the stratosphere and the effect of stratospheric processes on climate and weather.

This, however, hardly seems a field in which a joint flight is needed—surely as much would have been achieved if the Soviet team had made the results of their research available to the Americans? The only purpose for such a joint flight would be the joint calibration of two sets of instruments preparatory to a full programme.

● Some cooperative ventures, of course, clearly demand the participation of all partners. Thus the programme of seismic sounding of the Pamir-Himalaya fold, using charges of TNT exploded under the Kara-Kul' (USSR) and Attok (Pakistan) salt lakes, and at the foot of Mt Nanga-Parbat (India) is a clear case for participation by scientists of the countries concerned. During the 1974 tests all three countries were, indeed, represented. In the latest set of soundings (last month), however, a further parti-

cipant was added—Italy. It is difficult to find an explanation for this addition, other than at the diplomatic level. Italy is not particularly noted for work in seismic sounding and, to date, the principal Italian interest in the Himalayas has been that of mountaineering. The participation of individual scientists in the research projects of other countries, particularly when their own does not support a similar programme, has long been urged as a productive means of international cooperation in science. The Soviet system of centralised control of science means, in effect, that any such participation must be approved by the relevant government department, and therefore is apt to be presented to the world not as the small number of foreign scientists joining a given research team but as a major diplomatic issue.

● The influence of the Lysenko period in Soviet biology still, from time to time, appears in scientific and popular articles. One such echo of the past appeared in a recent *Pravda* story (August 25, 1975) on the "Party news" page, describing the heavily laden orchards attached to the Bryansk Machine-construction Plant. These orchards, it is claimed, "were originally planted with the participation of I. V. Michurin. The workers of the Bryansk plant turned to him for advice on how best to grow fruit trees." Michurin was, in fact, noted for his work on the acclimatisation of fruit trees, although many of his methods savoured of folk lore (watering the trees with sweetened water to teach them to bear sweet fruit, for example), but after his death in 1935, when Lysenko claimed him as a forerunner of his own peculiar views on plant selection, the name Michurin became synonymous with the anti-heredity school of biology. At a time when a new drive is being made in Soviet research towards investment of time, funds and talents in molecular genetics, the reappearance of the name Michurin, cited without explanation, as a kind of cult-figure to whom the success of the trees is attributed, strikes a somewhat odd note.

● The latest monitoring device recommended by Soviet experts on radioactive pollution in seawater is the sprat. According to the findings of an extensive study made in the Atlantic and off-shore seas of Europe, the sprat is highly sensitive to products of radioactive decay possessing a long half-life, notably caesium-137 and strontium-90. According to the research team, the sprat absorbs even microscopic admixtures of these isotopes quantitatively, with an accuracy comparable with that of the latest electronic equipment. □

BECAUSE of a failure on the part of its international Board of Management to agree on the future funding of the OECD Dragon High Temperature Reactor Project, formal steps will have to be taken early this month to run the project down. Formal, because nobody really thinks that an agreement will fail to be struck before notices to many of the 320 people who work on the project (most of them seconded from other organisations) expire in December.

Much of the blame for the delay, it seems, falls squarely on the British Department of Energy which is dragging its heels on the grounds that work on high temperature reactors (HTRs) has, as far as the UK is concerned, been overshadowed by the decision to go for the steam generating heavy water reactor as the basis of the next generation of nuclear power stations. The department is reluctant to continue contributing some £1.4 million a year to Dragon through the UKAEA, even though there is a considerable hidden bonus in that the UKAEA itself claws back £2.1 million a year from the Dragon Project (total budget £3.8

million a year) for services and so on provided at Winfrith, Dorset, where the Dragon reactor is situated. It is not clear that the overall costs of Winfrith could be cut by nearly £2.1 million a year if Dragon were to disappear.

Dragon has been running for 11 years during which time it has provided valuable operating experience of high temperature reactors both for the seven members of the project (six European countries together with Euratom) and for countries like the United States, where General Atomic, for example, is actively interested in commercial HTRs. Also, recently, there has been a surge of interest in the use of HTRs as sources of process heat for the chemical and steel-making industries. European steel-makers have even formed themselves into the European Nuclear Steel-making Club (ENSEC).

Clearly the UK's partners in the Dragon Project see it as a useful thing to persevere with for a further five-year period, though admittedly the UK itself might reasonably hope for some token reduction in the fairly large share of the bill which it picks up at present.

—Roger Woodham

W. C. FIELDS once remarked that when he visited Philadelphia, it was closed. The same remark could equally well be applied to Washington during the entire month of August this year. Congress has been out of action, President Ford and some of his top advisers have been taking things easy in Colorado, and many federal bureaucrats have been off on their summer holidays, leaving the tourists to face a heat wave and record-breaking pollution levels. The big news story has been whether or not oil prices will be decontrolled, and many esteemed journalists have been driven to writing about how dull it all is. Quite a change from last year.

But at least one body has been keeping busy. The International Cultural Foundation, an organisation linked to the Rev. Sun Myung Moon, a millionaire evangelist from South Korea, has been putting together a meeting called the "Fourth International Conference on the Unity of the Sciences", and a glittering array of scientific and academic talent has been signed up to take part in the event. The conference, which is set to take place at the Waldorf-Astoria Hotel in New York on November 27-30, is already generating some controversy.

A similar gathering was staged in London last year. It was also stiff with Nobel Laureates and it, too, generated considerable controversy because of Moon's association with the event.

Moon is the founder of the Unification Church, a religious cult founded on an anti-communist philosophy, and supported by street-corner fund raising and by the profits of several Korean industries. Moon, who is said to be close to the South Korean dictatorship, generated some attention last year when he led his followers in prayer on the Capitol steps for the political survival of President Nixon. And his church has recently been in the headlines thanks to a court suit brought by the parents of a teenage girl who are attempting to reclaim their daughter from its clutches. They claim that the church has so much psychological influence over her that she cannot exercise her free will.

The Fourth International Conference on the Unity of the Sciences has, however, attracted a list of sponsors and advisers which reads like a page out of the *Who's Who* of science. A letter sent with invitations to the meeting lists as chairmen or advisers Eugene Wigner, Alvin Weinberg, Kenneth Mellanby, Edward David, Werner Heisenberg, C. P. Snow, Jean Piaget, Julius Axelrod, Herman Kahn, Jonas Salk, and many others. But, according to an article in *Science and Government Report*, several of the participants listed in the letter are considering drop-

ping out. They were either unaware of Moon's connection with the International Cultural Foundation when they accepted invitations, or they are simply having second thoughts about being associated with an event sponsored by Moon's organisation. Similar problems arose with the London meeting last year.

Discontent about Moon's sponsorship of the conference has prompted section chairmen to send a letter to other participants assuring them that the International Cultural Foundation will not interfere in the conference, and that participants will be free to express their own views.

Washington seen

by Colin Norman



But the letter hasn't stilled the discontent and more dropouts can be expected.

● Congress reassembles this week, prepared to do battle with President Ford on oil prices and various other matters. In between the skirmishes, it may get round to passing a bill to re-establish a science policy office in the White House. There has, at least, been some discernable progress on that measure.

Last month, on the eve of the Congressional recess, Representatives Olin Teague and Charles Mosher, respectively the chairman and senior Republican on the House Committee on Science and Technology, jointly introduced a bill which is likely to be passed by the House with little change. The measure is similar in many respects to President Ford's proposal, although it spells out the duties of the office in some detail, and it is prefaced by a

lengthy discourse on the objectives for a national science policy.

The bill would establish a White House Office of Science and Technology Policy (OSTP), headed by a director who would double as the science adviser to the President, and four deputies. Its chief task would be to provide advice to the president on "scientific and technological considerations involved in areas of national concern, including, but not limited to, the economy, national security, health, foreign relations, the environment, and the technological recovery and use of resources. It would also be required to help in the preparation of the federal budget, and "assist the President in providing general leadership and coordination of the research and development programs of the federal government". The bill does not state how the office should function within the White House, however, leaving such arrangements for the President to determine.

One item in the bill which was not included in Ford's proposal is the setting up of a Presidential commission, consisting of between 5 and 12 scientists, to carry out a 15-month study of the federal government's science and technology programmes, and to recommend ways in which the overall effort can be improved.

The bill is expected to be passed by the House Science and Technology Committee later this month, and by the full House in October. Meanwhile, the Senate Committees on Labor and Public Welfare, Science and Astronautics, and Commerce, which all have jurisdiction over the bill, will probably hold hearings later this month.

One factor which could upset the timing however, is that Teague was taken ill last month with what was believed to be a stroke, and although he has been discharged from hospital, it is unclear whether his committee schedules will be affected.

● The National Science Foundation (NSF) which has been having severe political problems with critics in Congress, has so far been let off lightly. An amendment designed to give Congress power to veto any individual NSF grant before it is awarded, which was attached to a bill earlier this year by the House has finally been discarded completely. The final version of the bill was passed without the amendment last month and sent to President Ford. ● Finally, the Atomic Safety and Licensing Board has demonstrated a fine sense of irony. It has scheduled a hearing on the proposed demonstration liquid metal fast breeder reactor, which has broken all records for cost overruns, in the US Bankruptcy Courtroom.

correspondence

Spoon bending: an experimental approach

SIR,—We have investigated six young people who claimed the power of bending objects by stroking in the manner demonstrated on television recently by Uri Geller and others. In this report we will call these people A, B, C, D, E, and F. A, B, and F are young girls all aged eleven years. C is a girl of thirteen, while D and E are boys aged ten and eight respectively. All were contacted with the aid of local press and television. A, B and C had received publicity in the local evening papers. Subsequently Dr Pamplin appeared on BBC "Points West" local television news programme when B demonstrated her ability quite convincingly. The parents of D, E and F subsequently contacted Dr Pamplin claiming that their children could also bend cutlery by stroking.

Most of the subjects were first visited in their own homes where they showed their ability in the casual atmosphere of their sitting rooms. A succeeded in bending a weighed and measured rod of mild steel of 3/10ths inch diameter supplied by the experimenters as well as her own cutlery.

All six subjects were subsequently tested in Bath University's psychology laboratory. This laboratory has three large one-way mirrors behind which the experimenter can observe, photograph and take television video tape unseen by the subject. In all cases, except A, one or more observers sat in the laboratory with the subject. In the first four experiments there was a second television camera in the laboratory with the observers. The subject was handed the spoon or rod after its outline had been drawn on a sheet of paper and was allowed to stroke it in the approved manner between forefinger and thumb of one hand and to report what they felt was happening. B reported that the spoon "felt soft" before bending. C stated that it "felt like plaster, then running water". The running water feeling occurred, it was said, just before bending occurred. However, at no time did C bend anything for us while experimenters were watching. The others all succeeded in bending spoons and B bent a rod of mild steel as well.

The aim of the experiment was to obtain a photographic or video taped record of the actual moment of

bending. The observer in the room measured the spoon against its outline at intervals during the session noting the time.

The observers in the room were instructed to deliberately relax their vigilance at intervals after the first twenty minutes. The experimenters were specially alert during these periods and in all cases except C they observed and photographed cheating by the subjects. A put the rod under her foot to bend it; B, E and F used two hands to bend the spoon using considerable muscular power, while D tried to hide his hands under a table to bend a spoon in both hands out of sight of the observer.

We can assert that in no case did we observe a rod or spoon bent other than by palpably normal means. We cannot, of course, conclude that all instances of the so called Geller Effect are due to cheating. However we offer details of our experimental procedure in the hope of helping other experimenters design experiments that can be used when cheating is suspected.

DR BRIAN R. PAMPLIN
MR HARRY COLLINS

Bath University, UK

Presto! Would you believe?

SIR,—In his review of John Taylor's book, "Superminds", Dr C. Evans comments on the ease with which the author seems able to discount the possibility of fraud and deceit on the part of the children involved in purported "psychic" performances. I recently launched an experiment with a newspaper which reports psychic phenomena to prove that persons lacking expertise can easily be deceived by a person using very simple methods—if the predisposition to belief is there. I am a professional conjuror, and needed only modest skills to convince the newspaper that I was indeed possessed of very strong paranormal abilities. It was only necessary to claim genuine powers for the observer to relax completely and believe all.

The need to have a competent conjuror present at performances of this nature is very evident. Otherwise, the observations are of no value scientifically.

JAMES RANDI

51 Lennox Avenue,
Rumson,
New Jersey

Publishing problems

SIR,—I wish to draw your attention to the difficulties which Czechoslovakian scientists seem to be encountering when publishing in international journals.

From a letter which I received from one of our contributors I learn that a paper sent to him arrived in Prague on April 7, 1975, but the author did not receive it until May 23, 1975. Furthermore, he was "commanded out" of Prague on May 24 for two weeks.

I am wondering what these ominous words "commanded out" actually mean. As fellow scientists, we have all been deeply conscious of the treatment received by some of our colleagues in Russia, who have been "frozen out" or even interned.

Let us hope that the Czechoslovakian government will not resort to Soviet methods and will continue to allow their talented scientists to publish in international journals.

JØRGEN CLAUSEN

Neurobiology,
Copenhagen, Denmark

Additive safety

SIR,—“We tend to trust Mother Nature, and suspect the organic chemist”, says Thomas Jukes mockingly in his vivid defence of synthetic food additives August 7. There is, of course, a reason to trust natural food better than food additives which must be familiar to the author of *Molecules and Evolution*: Organic chemistry happens to be 147 years old; man is at least 1×10^6 years old, and has thus fed for 999,853 years on natural food. The fact that man has survived all these effects of “numerous chemicals (in natural food) that are all more or less toxic” does not prove at all that there will be some fittest to survive the ingestion of at least as many food additives introduced mostly in the past 10-20 years.

Indeed, we do not know whether sodium benzoate has some disagreeable effects within 3 or 10 generations, whereas sugar (“one of the most ancient and universal of food preservatives”) has passed the most rigorous toxicological test there is: time. Besides, sugar tastes better.

R. E. HUMBEL
University of Zurich, Switzerland

news and views

STARTING, paradoxically, with invisibly faint electron micrographs of purple membrane patches from *Halobacterium halobium*, Nigel Unwin and Richard Henderson have reconstructed the first distinct electron images of the polypeptide chain packing inside a protein molecule (see page 28 of this issue of *Nature*). This breakthrough at the MRC Laboratory of Molecular Biology in Cambridge is a dramatic culmination of the electron micrographic "crystallography"—or Fourier microscopy—developed there by Aaron Klug and his colleagues over the past decade.

Unwin and Henderson's technique opens up a new frontier in the exploration of the internal structure of proteins—particularly those which build periodic cellular assemblies unsuited for X-ray diffraction crystallography. No one name has yet been devised to identify their method but it can be described as high-resolution, low-dose, under-focus phase-contrast, three-dimensional electron microscopy of thin, unstained, periodic biological specimens.

Drying, staining and electron irradiation can pretty well destroy the molecular order in proteins. Until now significant detail finer than about 20 Å in biological structures has rarely been revealed by electron microscopy even though good microscopes can give 3 Å point-to-point resolution. Unwin and Henderson have mitigated the trauma of dehydration on their specimens by the clever trick of replacing the water with glucose syrup (*J. molec. Biol.*, **94**, 925; 1975). Electron diffraction patterns from their sweetened purple membranes and thin catalase crystals demonstrate that periodic order is preserved out to 3.5 Å spacing, as long as the radiation dose is less than about 0.5 electrons Å⁻². They have obtained micrographs at 7 Å resolution by leaving out the stain which eliminates the usual source of contrast, and by cutting down the electron dose, which raises the noise level well above the signal. Fourier transformation is crucial for correcting, averaging and recombining the invisible microscopic images.

Contrast is produced in the micrograph of the transparent object by underfocusing. But this phase contrast oscillates between positive and negative with increasing spatial frequency, so compensation is essential to recover an undistorted image. The focus is restored using the Fourier processing

Distinct images from invisible micrographs

from D. L. D. Caspar

method previously applied to negatively stained specimens: the computed Fourier transform of the featureless low-dose micrograph—which shows sharp diffraction maxima from the regular lattice—is divided by the modulation transfer function to obtain the correct phases of the object with maximum contrast. The nodes of the transfer function are measured from the optical diffraction pattern of a high-dose micrograph which displays the contrast oscillations although the specimen itself has been incinerated.

At the safe dose for the 7 Å resolution image, only about 25 electrons on the average pass through each 7 Å × 7 Å square of the specimen. Since the difference in the number of electrons forming dark and light regions of the image at maximum phase contrast is less than 1%, the local statistical fluctuations in the micrograph are much larger than the signal. The weak signal from the periodic object is extracted from the noise by averaging over some thousands of unit cells. Evaluation of the corrected Fourier transform at the reciprocal lattice points and retransformation effects this averaging to give an in-focus projected image of a single unit of the real lattice. This is the essence of the optical filtering method: the larger the number of regularly arranged units in the lattice, the sharper the diffraction spots and the weaker the periodic signal in the image which can be distinguished from the noise whose transform extends throughout reciprocal space. The intensity of the spots is actually measured from the electron diffraction pattern of the periodic specimen, but the indispensable phase information is obtained by computation of the compensated Fourier transform of the low-dose, under-focus image.

Tilting the purple membrane lattices provides projected views from different directions which are recombined using the Fourier method of three-dimensional image reconstruction. Correcting for defocusing from large tilted specimens and refining the phase calculations to the same origin for

different views presents special problems which have been resourcefully solved by Unwin and Henderson. The maximum angle of tilt for these micrographs is 57° which leaves about 37% of the 7 Å sphere in reciprocal space unsampled. Fortunately, the amplitudes of the Fourier components in the missing regions are small. Thus, their 7 Å resolution map provides an accurate representation of the α -helical rods extending roughly perpendicular to the plane of the membrane, although the short connecting segments parallel to the surface are not yet seen. This remarkable three-dimensional picture illuminates both the objectives and the methods of structural biology.

In order to see inside protein molecules sharply at high resolution, Unwin and Henderson record very faint, out-of-focus micrographs of large periodic arrays at relatively low magnification. This "coincidence of contraries" reflects the antithesis of "distinct images and clear conceptions" recognised by S. T. Coleridge in a more epistemological context. The concepts for dealing with periodic images in real space have been most coherently formulated in reciprocal space in the 60 years since W. H. Bragg first pointed out the utility of Fourier synthesis for crystal structure analysis. Fourier transformation is not the only way to recover information about three-dimensional structure from electron micrographs but it has been, so far, the constructive way.

The development of Fourier microscopy of biological structures in Cambridge recapitulates in many ways the history of X-ray crystallography started there by W. L. Bragg in 1913 and, more particularly, protein crystallography begun there by J. D. Bernal and Dorothy Crowfoot Hodgkin in 1934. By the early 1950s Max Perutz and W. L. Bragg were mapping the outline of proteins by tracing the molecular transform and by using a kind of negative staining for X-ray diffraction; the internal structure of proteins became visible with X-rays in 1953 when Perutz successfully applied the isomorphous replacement method to solve the phase problem.

Electron micrographic crystallography was begun in the mid-1960s by Klug and his colleagues using optical diffraction and by 1968 it had become Fourier microscopy using computation of Fourier transforms of digitised images for the reconstruction of three-dimensional structures. There

is no "phase problem" in this microscopy since the phase information is contained in the image—but there was a "specimen problem" since the images, at best, showed little more than the shadow of the stain encasing the biological molecules. Unwin and Henderson have now introduced an ingenious but simple technique for solving the specimen problem which makes it possible to see inside molecules with electrons, perhaps to the ultimate limit of resolution of the microscope itself.

Any thin periodic structure with large ordered domains that can survive the extreme environment inside an electron microscope is a suitable object for imaging by this method. Their approach will be very useful for analysing the structure of molecules, such as lipids and some proteins, that form thin crystals difficult to study by X-ray diffraction. The unique power of the method for determining the structure of molecules that build cellular "surface crystals" is beautifully demon-

strated by Unwin and Henderson's image reconstruction of the purple membrane.

The purple membrane was isolated seven years ago in Walther Stoeckenius' laboratory as a pigmented by-product of studies on some other curious structures from this halophilic bacterium; it has now become an object of central importance in membrane biology. Photons absorbed by the chromophore, retinal, pump protons across this specialised membrane assembly. Analysis of the operation of this system is clarifying basic mechanisms in vision, photosynthesis and oxidative phosphorylation. Unwin and Henderson's image of the structure is comparably informative. The interlocking of the X-helical segments of the purple membrane protein, as in Crick's coiled-coil, and the fitting of the lipid bilayer in the interstices of the protein lattice illustrate key aspects of the structure of structural proteins and the organisation of membranes.

Towards universal geothermal power

from Peter J. Smith

"We are, with regard to this problem of utilizing the Earth's internal heat, very much in the position of an eighteenth century 'outcropper' quarrying for coal in the hillsides of Yorkshire. Noting the dip of the strata, he could quite well reason that far below the surface there must be immense reserves of coal, which on the one hand he did not need to exploit because the hillside quarry provided for all his immediate needs, and which on the other he could see no possibility of exploiting at a profit."

These words were spoken by John L. Hodgson at the British Association meeting in Leeds 48 years ago this week; but a similar statement could almost have been made today. The only difference is that the more generalised mid-20th century version of the 'hillside quarry'—cheap and abundant fossil fuel—is no longer with us. It disappeared so recently, however, that until now there has been little incentive to tap the "vast potentialities" of geothermal energy that Hodgson dreamt of.

Indeed, the whole subject of the Earth's internal heat has developed remarkably slowly from its origins in the 17th century. The earliest known reference to high temperatures within the Earth is that by Morin (*Nova Mundi Sublunaris Anatomia*, Paris, 1619) who found the air becoming warmer as he penetrated deeper into a

group of Hungarian mines. Some 50 years later Robert Boyle recorded (others') eyewitness accounts of temperature increases in mines and discussed the source and transport of underground heat, concluding that heat of chemical origin from the Earth's deep interior rises by conduction and the movement of material to produce the observed near-surface increase of temperature with depth.

From 1868 to 1883 a committee of the British Association pursued the subject vigorously and systematically and, having finally appreciated that the determination of heat flow requires the measurement of thermal conductivity as well as temperature gradient, obtained a mean heat flow of $1.3 \mu\text{calorie cm}^{-2} \text{s}^{-1}$, a value only slightly lower than today's average based on many more data.

But although 'academic' studies of heat flow are now beginning to contribute to the search for sources of geothermal power, the practical use of the Earth's internal heat (which has a much longer history) apparently proceeded quite independently, largely on the basis of chance discoveries of particularly conspicuous and high local concentrations of near-surface heat.

On the industrial front, the Italians have been extracting boric acid from natural steam jets at Larderello in Tuscany since the late 18th century. Historically, however, the Larderello

site is even more significant since it was there that geothermal power was first produced successfully (in 1904) when steam was piped to low pressure turbines driving small electrical generators. The dry (superheated) steam power stations at Larderello still account for about 40% of the world's geothermal power output, although there is now also a wet steam power station complex at Wairakei in New Zealand, a dry steam system at The Geysers in California and smaller stations in Japan, Iceland, Mexico and the USSR. The total geothermal power capacity installed throughout the world is now about 1,000 MW, which is still an insignificant proportion of the total power from all sources. In addition to power generation, however, geothermal steam is also used directly in various parts of the world for paper making, sewage treatment, potash mining, district space heating, growing greenhouse crops in cold climates and for many other purposes which can be effected close to the geothermal sources themselves.

The variety of uses for geothermal heat is impressive even without adding proposals in the pipeline. But as Morton C. Smith of the Los Alamos Scientific Laboratory pointed out last year at a meeting of the American Institute of Physics, the development of types of sources currently in use, especially for power generation, poses numerous as yet unresolved problems. For one thing, dry steam reservoirs such as those at Larderello and The Geysers seem to be extremely rare. There are fewer than a dozen known 'vapour dominated' geothermal fields of possible commercial size throughout the world, the largest of which would be capable of development to no more than 3,000 MW. It is possible, of course, that there are many dry steam sources which remain undiscovered because they have no surface indicators; but if so, the chances of finding them quickly are small because remote sensing systems capable of detecting subterranean steam are still poorly developed. Moreover, there are many scientific and technical unknowns. Is a dry steam reservoir a wasting or self-renewing asset? What are the interactions between heat flow, fluid flow, mass transport and tectonic stress changes? How can minerals (silica, for example) deposited from the steam be prevented from clogging up the works and volatiles (for example, hydrogen sulphide and boron and arsenic compounds) be prevented from causing pollution? Notwithstanding operating experience to date, none of these questions has been completely answered.

Natural geothermal systems in which the fluid is mainly hot water are much more common than those containing

predominantly dry steam; but the problems of power evaluation, development and production here are even greater, largely because of dissolved minerals. The natural pressures in most hydrothermal reservoirs are too low to maintain artesian flow, so the water must be pumped to the surface. But the hot brines rapidly destroy drilling equipment, borehole casings, pumps, valves and the rest of the hardware required to bring the fluid to the surface and extract heat from it. And once the heat is extracted the residual highly-concentrated brines then pose waste (thermal and chemical) disposal problems. The removal of large quantities of subsurface fluid can also lead to extensive subsidence. According to Smith, therefore, "the widespread exploitation of a large and readily available geothermal resource will evidently be delayed for some years until economic solutions to [these problems] have been demonstrated".

Where, then, does the long-term future of geothermal energy exploitation lie? The answer favoured by the Los Alamos group and currently being investigated by them is the extraction of heat from dry rock. Even in steam and water reservoirs well over half of the thermal energy resides in the rock, and rocks at depth are hot even when fluid is absent. In principle, therefore, it should be possible to extract geothermal heat anywhere in the world as long as sufficiently high temperatures are reached at depths to which drilling is economic.

Although it is seldom, if ever, acknowledged, the simplest way of achieving this dream was described in some detail by Hodgson in his talk in 1927 when he proposed an artificial version of the hydrothermal system that nature itself rarely provides. In other words, he suggested that cold water should be passed through the hot rock at depth and then recovered in the form of hot water or steam. This is almost exactly the method now adopted by the Los Alamos group with the difference that, whereas Hodgson envisaged a closed pipe circulation, the modern version has the injected water making direct physical contact with the hot rock and rising through a second borehole. An obvious unknown in either system, however, is whether there is an adequate area of contact at depth to achieve sufficient rock-to-water heat transfer. Hodgson's solution was to increase the length of his pipe at the bottom of the borehole if necessary; the modern idea is to fracture the rock at depth.

But whereas Hodgson's proposal was destined to remain unrealised, the Los Alamos suggestion is actually being put to the test at a site in New Mexico. According to Laughlin (*Geotimes*, 20,

March 1975), the main hole has already been drilled to a depth of 2,929 m in impermeable rock where the bottom-hole temperature is 196 °C. Hydraulic fracturing of the surrounding rock was successfully carried out at 1,981 m and will be repeated at the bottom. A second, somewhat shallower, hole is then to be drilled into the lower fracture zone to act as the hot fluid ascent route.

If the dry rock experiment in New Mexico proves scientifically and technically successful and an economically viable geothermal heat extraction system results, the way would clearly be open to a staggering increase in the use of this widely available and relatively pollution-free energy resource. Presumably it is still far too early to say when, or even whether, this ideal state will ever be reached. In the meantime, however, it must be evident that, other things being equal, both science and economics would benefit from application to areas where the heat flow is relatively high and the thermal energy is close to the surface.

The high heat flow anomaly in the Gulf of California, whose discovery is reported by Lawver *et al.* on page 23 of this issue of *Nature*, fulfils both of these criteria, having a remarkably high maximum value of 30 $\mu\text{calorie cm}^{-2} \text{ s}^{-1}$ (which compares with 4.5 $\mu\text{calorie cm}^{-2} \text{ s}^{-1}$ at the New Mexico site) and coming from a basaltic intrusion within 100 m of the surface. Admittedly the heat source is not continental; nor is it entirely clear to what extent, if any, hydrothermal fluids are involved. What is certain, however, is that Lawver and

his colleagues have discovered one of the Earth's major geothermal resources and that it is in just such areas that the real case for geothermal power will be fought. □

X-ray sources and the ionosphere

from L. J. C. Woolliscroft

THE lower ionosphere is a complex region of the upper atmosphere, with several ionisation mechanisms and high enough neutral species densities to cause many ion and electron reactions with the weakly ionised plasma. The production of ionisation by non-solar sources has been calculated by, for example, Velinov (*J. atmos. terr. Phys.*, **30**, 1891; 1968) for the influence of galactic cosmic radiation on the bottom of the D-region. More recently other non-solar objects have been invoked as sources of ionisation in certain conditions.

The propagation of low frequency (LF) and very low frequency (VLF) radio waves from extremely stable transmitters such as those used to broadcast atomic time allows the bottom of the ionosphere to be monitored continuously. Small changes in the phase and amplitude of the received LF and VLF signals from distant transmitters are used and some of these have been associated with the meridian transit of a strong X-ray source. Edwards *et al.* (*Nature*, **222**, 1053; 1969) found a nocturnal VLF effect from Scorpius XR-1 and the same source was found to give an effect at LF by Ananthakrishnan and Ramanathan (*Nature*, **223**, 488; 1969). Later positive results have included other celestial X-ray sources and also flare effects in Sco XR-1.

That these radio propagation effects are due to X-ray sources was challenged by Poppoff and Whitten (*Nature*, **224**, 1187; 1969) who showed that the ionisation production rate was at least an order of magnitude lower than that due to other mechanisms, especially that of Lyman α radiation ionising nitric oxide. A difficulty exists, however, in calculating the production of ionisation by Lyman α because the concentration of NO is somewhat uncertain.

A further complication comes from the masking effect of precipitating electrons producing ionisation at other than low latitudes which may account for some of the negative results such as those of Burgess and Jones (*Nature*, **224**, 680; 1969).

In this issue Karszenboum and Gagliardini (page 34) have calculated the production rate of ionisation due to several galactic X-ray sources using more recent spectra which extend to



A hundred years ago

PROFESSOR PALMIERI has discovered a new instrument which he calls a "diagometer," and which is constructed for the rapid examination of oils and textures by means of electricity. What the apparatus will do, Prof. Palmieri details thus:—1. It will show the quality of olive oil. 2. It will distinguish olive oil from seed oil. 3. It will indicate whether olive oil, although of the best appearance, has been mixed with seed oil. 4. It will show the quality of seed oils. 5. Finally, it will indicate the presence of cotton in silken or woollen textures. The professor has been complimented for this invention by the Chamber of Arts and Commerce at Naples, who have published a full description of the apparatus, with instructions for use.

from *Nature*, **12**, 427; September 9, 1875

higher energies and new data for the photoionisation yields. They compare their production rates for X-ray sources with the nocturnal production by other mechanisms and conclude that at least Sco XR-1 and probably some others could be significant at heights around 80 km.

The daytime production has to compete with solar X rays and ultraviolet, making measurements less easy. Ramanamurty *et al.* (*J. atmos. terr. Phys.*, **32**, 1721; 1970) found an amplitude variation over the LF path from Tashkent to Delhi at the time of an X-ray flare from Sco XR-1 and argued that this was theoretically possible under favourable geometry conditions with certain values for the NO concentration.

If the electron density of the D-region is enhanced by an X-ray source then the conductivity will also increase altering the pattern of ionospheric currents and thus the magnetic field. Murty and Yacob (*Planet. Space Sci.*, **22**, 1583; 1974) have studied the average sidereal time variation of the nocturnal geomagnetic horizontal component and found perturbations at the times of transit of some of the stronger X-ray sources. There is some doubt about their results since the magnitude of the perturbation does not correlate with the strength of the source as one would expect from a simple mechanism, indicating perhaps contributions from other effects.

The first daytime perturbation in the magnetic field is reported by Sastri and Murthy on page 35 of this issue, using magnetometer data during the same Sco XR-1 flare as Ramanamurty *et al.* Unfortunately a small solar X-ray flare occurred 33 min before the magnetic field perturbation, but Sastri and Murthy argue that this was both too weak and too early to cause the effect. The detailed mechanism for these geomagnetic effects remains uncertain but will probably involve a pattern of ionospheric current similar to that at the time of a solar flare.

The use of this ionospheric technique to study other astronomical sources such as γ -ray bursts is being studied by Mongain and Baird (paper A.2.7 presented at Cospar, Varna, June 1975). They argue that for the detection of γ bursts the ionospheric recombination time is very important and the best time may not be at night when the recombination time is short compared with the burst, but during the day. The analysis of their data is currently proceeding but they feel that as yet the work will tell us more about the ionosphere than about astronomy. The recently reported strong transient X-ray source A0621-00 discovered by the satellite Ariel V may also serve as a test for many of the theories. □

Primary, secondary, tertiary

from E. G. Richards

WHAT is the nature of the intercistronic regions in *Escherichia coli*? Platt and Yanofsky (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2399; 1975), have recently provided us with an answer in the case of the junction between the B and A cistrons of the *trp* operons. Making use of the high yield of the distal end of the corresponding messenger RNA from the *trp*ALD102 mutant and the fact that DNA from phage λ trpB27 and ϕ 80trpA37 carries sequences complementary to the end of the B and the start of the A cistron (and hence will hybridise with the messenger and protect it from nuclease attack in this region), they were able to isolate a fragment of 32 P-labelled RNA about 200 bases long. This was fingerprinted and a comparison of the oligonucleotides produced with the known sequence of the two proteins enabled them to determine most of the sequence. Any doubts about the part between the two translated regions were resolved by fingerprinting and sequencing the shorter polynucleotides produced by binding the longer piece to ribosomes to form the initiation complex of the A cistron and trimming the ends with nucleotides. The final result is of no little interest in that the termination signal for the B protein is found to be a single UGA codon which is out of phase with the AUG initiation triplet for the A protein; indeed, the final A of the termination codon is the initial A of the initiation codon, so that there are only two bases which are not translated. A consequence of this is that if the initiation recognition site of the A protein involves more than this UGAUG sequence it must also be translated as part of the B or A cistron. The authors point out that this and other known instances of dual function in mRNA provide some rationalisation for the degeneracy of the genetic code. They also note that close to the end of the B cistron and within the A cistron ribosomal binding site is a sequence GAGGGG which could bind to the CUCCUU sequence at the 5' end of the 16S rRNA; an interaction between such regions has been proposed by others.

Platt and Yanofsky do not speculate on possible secondary structures of their messenger fragment but such cogitations have been taken up by Pipas and McMahon (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2017; 1975) albeit for tRNA sequences rather than RNA. The consistency of the clover leaf structure with all known tRNA sequences as well as with recent X-ray

crystallographic investigations has probably convinced all but the most hardened sceptic of its reality. Nevertheless it is still valid to ask if the clover leaf structure is the structure with minimum free energy. Several authors have published descriptions of computer programs that will list all possible base pairing schemes compatible with a given sequence and other workers have provided, on the basis of work with model compounds, formulae for assessing the free energy of formation of secondary structures (from the random coil) in terms of contributions from various features such as loops, bulges and helical regions. Pipas and McMahon have put these two notions together and written a program that gives the free energy of all possible structures. They begin, as have others before them, by getting the computer to produce a list of all possible helical regions containing at least three base pairs. GU pairs are allowed but not at the end of a helical region; the hairpin loop must contain at least three bases and there are one or two other restrictions of a similar nature. The next step is to decide whether a given pair of helices are compatible or not, that is, whether they co-exist in the same structure. Clearly two helices that involve the same bases are incompatible and the authors also assume that if the bases in the hairpin loop of one helix participate in the formation of another helix, they only do so by pairing with other bases in this same loop. This latter restriction means that only structures resembling clover leaves are considered and more complicated patterns of folding (which cannot be drawn on a sheet of paper without the chain crossing itself) are not considered. This is probably the greatest weakness in their results but was required at the present state of play by the fact that the data for assessing the free energy of these more complicated structures are at present unknown. The next stage in the calculation was to enumerate one by one all the possible sets of compatible helices and then to work out the free energy of each.

Sixty-two tRNA sequences were presented to this program on a CDC6500 computer and all were analysed in less than 3 minutes. It was thus found that for 32 of these sequences, the clover leaf had the minimum free energy. Of the remainder, the minimum free energy structure of 13 corresponded to clover leaves with one or other (usually the DiHU) of the stems undone, and in five sequences the conventional acceptor stem contained non-standard base pairs which led to its elimination by the rules described above. This left 12 sequences which gave more extended structures as the most stable,

but their free energy was within 5 kcalorie of that of the clover leaf. Several explanations are available for the anomalies: the thermodynamic data on which the energy assessments are based may be faulty in some way or the native clover leaf form may be trapped in a metastable state; but the authors incline to the notion that tertiary interactions may tip the balance in favour of the clover leaf, and in the worst case only 5 kcalorie would be required.

The experimental elucidation of such tertiary interactions by NMR techniques is the subject of an interesting paper by Reid *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2049; 1975). They determined the NMR spectrum of several highly purified species of tRNA in the -9 to -15 p.p.m. region. The -11 to -15 p.p.m. region gives a signal believed to emanate from the hydrogen-bonded ring NH protons and proportional to the number of Watson-Crick base pairs. Nevertheless it has been suspected that this low field region should contain resonances due to other sorts of base pair and to tertiary interactions. Their first interesting observation was that tRNA^{Val}, tRNA^{Arg} and tRNA^{Phe} all from *E. coli* gave a resonance corresponding to two protons at about -10.5 p.p.m., whereas tRNA^{Asp} from yeast gave a signal corresponding to about 10 protons in this region. The three *E. coli* sequences all indicate one GU base pair somewhere in their clover leaf structures while the yeast sequence indicates no less than three GU pairs and a GΨ pair as well. Since a GU pair involves two ring NH protons hydrogen bonded to oxygen atoms, the authors tentatively assign the -10.5 p.p.m. resonance to GU pairs.

The next point concerns a single proton resonance at -14.9 p.p.m. observed with these same three *E. coli* species but absent in several yeast tRNA spectra. For these *E. coli* species ring current shifts were not expected to result in any resonance below -14.5 p.p.m. Now the *E. coli* sequences all have s⁴U in position 8, whereas yeast tRNA^{Phe} has an unmodified U in this position which the X-ray diffraction data suggest is hydrogen bonded to A14. This suggested the attractive hypothesis that the -14.9 p.p.m. resonance corresponded to a S⁴U8-A14 base pair. This was neatly confirmed in the case of *E. coli* tRNA^{Val} by removing the sulphur by the action of cyanogen bromide. When this had been done, the tRNA was still active in aminoacylation (and hence presumably still had the native tertiary structure) but the -14.9 p.p.m. resonance had gone.

Other resonances in the -11 to -15 p.p.m. region probably remain to be

defined and assigned, because in the case of *E. coli* tRNA^{Val}, the integrated intensity from this region corresponds to 26±3 protons. One of these is, no doubt, the s⁴U-A tertiary interaction and 20 more no doubt correspond to the 20 Watson-Crick base pairs in the structure, but this leaves several more unaccounted for. The authors also suggest it likely that resonances corresponding to four protons in the -9 to -10 p.p.m. region in the case of *E. coli* tRNA^{Val} should be assigned to amino protons hydrogen bonded to ring nitrogens. Several such hydrogen bonds appear in the tertiary interactions discerned in yeast tRNA^{Phe} and *E. coli* tRNA^{Val} has corresponding bases in corresponding positions of the sequence.

Clearly more work needs to be done to confirm some of these tentative assignments and to elucidate others but this technique is reaching the point where it can provide information concerning the three dimensional structure of nucleic acids which is almost on a par with that obtained from X-ray crystallography. □

Ectoparasite provides cercarial model

from F. E. G. Cox

DIGENETIC flukes typically use two hosts during their life cycle, a mollusc and a vertebrate. The larval stage infective to the vertebrate is the cercaria which passes out from the snail and often has a short free-swimming existence before it gains access to its final host. Cercarial activity and infectivity have been studied in economically important species such as *Schistosoma mansoni* and *Fasciola hepatica* but it has been difficult to obtain much quantitative information from these studies because of problems such as having to dissect the final host to find out how many cercariae have actually established themselves. Because these problems are inherent in dealing with any internal parasite there has been no particular interest in developing alternative laboratory models and the study of the digeneans has therefore tended to suffer.

A convenient model is now available and it is an ectoparasite so easy to maintain that it will obviously become very important in the future. This fluke is *Transversotrema patialensis* and it lives under the scales of many freshwater fish including *Brachydanio rerio*, the Zebra Danio, which is sold

by many shops stocking tropical fish. The snail host is *Melanoides tuberculata* which is also available from aquarists. When infected fish are put in a tank containing uninfected snails the life cycle maintains itself indefinitely and the tank provides a convenient source of every stage required for experimental investigations. It is also easy to see how many adult flukes have become established simply by looking at the fish alive.

Transversotrema patialensis is therefore an ideal subject for the study of cercarial survival, activity and infectivity. Anderson and Whitfield (*Parasitology*, **70**, 295; 1975) have found that cercariae survive for a maximum of 44 h and that infectivity is restricted to half this time. As these cercariae do not feed they can live only as long as their energy reserves persist; Anderson and Whitfield have produced theoretical models relating infectivity to activity and activity to the amount of glycogen available and have found good correlations between their models and their experimental observations. They used electron microscopy as well as the more usual histochemical techniques in order to observe the depletion of glycogen in the cercariae.

Because the period of infectivity is so short the cercariae must locate, identify and attach to an appropriate host as quickly as possible. In the logical follow-up to the first study Whitfield, Anderson and Moloney (*Parasitology*, **70**, 311; 1975) were able to conclude that the location of the fish is a random process but that essential information regarding the suitability of the host is probably obtained by the use of receptors on the tail of the cercaria. Scanning and transmission electron microscope studies show that these receptors are unique but similar to types found in monogeneans, also ectoparasites, and which are thought to be concerned with chemoreception. After recognition of the host, attachment is accomplished first by means of adhesive pads, also on the tail, and then by the ventral sucker on the body after which the tail is lost and the fluke attains its final position under a scale.

These experiments open up a whole new field of investigation into the biology of flukes because the number of flukes that have actually established themselves can be counted without the need for killing and dissecting the host. These two papers are only a beginning but the standard set should ensure that further studies will also be based on critical experimental observations coupled with the use of techniques such as electron microscopy and mathematical analysis when necessary.

Solar oblateness

from David W. Hughes

THE Sun is thought to be flattened. Dicke and Goldenberg (*Astrophys. J. Suppl.*, **27**, 131; 1974) gave $(4.51 \pm 0.34) \times 10^{-5}$ as the value for this oblateness which means that the polar radius is 31.4 km smaller than the equatorial radius. Not much considering the mean solar radius is 695,997 km. Now it is clear that a rapidly rotating solar core with a period of between 0.5 and 2 d could provide an explanation for this flattening; but what is not clear is why the core should rotate so rapidly. This problem has been approached theoretically in a recent article by Schatten (*Astrophys. Space Sci.*, **34**, 467; 1975).

Obviously there must be some interaction between the quickly rotating core and the slowly rotating photosphere. Because the solar interior contains essentially all the mass and the angular momentum the photosphere would be spun up to the angular velocity of the interior unless there were some braking force acting on it. Dicke suggested that it is the accelerating torque exerted on the solar wind by the magnetic fields locked in the photosphere which provide this brake. In his first approach to the problem Schatten assumes that the rate of change of angular velocity with time is negligible and then finds that the angular velocity of the solar core is simply obtained by dividing the solar wind's torque, τ_{sw} , by the rate of change of the solar moment of inertia with time dI/dt . This moment of inertia varies because the nuclear reaction in which hydrogen is converted into helium reduces the number of ionised particles in the solar interior. To conserve the number of particles per unit volume and the pressure balance the core has to contract. Taking τ_{sw} as -8×10^{30} dyne cm, calculations of dI/dt as a function of r , the distance from the centre of the Sun, gives a rotation period of 17.5 h for $r < 117,000$ km and 25.4 $(r/R_{\odot})^2$ days for $r > 117,000$ km.

In Schatten's second approach the square of the angular velocity of the core is found to equal the mean power loss due to solar activity, P_{sa} , divided by $0.5 dI/dt$. As the core contracts the rotation power is liberated through the magnetic fields in sunspots. The energy lost in a solar cycle is calculated to be 10^{35} erg, equivalent to 2×10^{27} erg s^{-1} . However some of the spot energy might not get away from the Sun and Schatten, considering flares, gets a more dependable value of 10^{28} erg s^{-1} . These two values give a core rotation period of between 1 and 4 d. Third, the core rotation period must equal P_{sa}/τ_{sw} which gives periods of 0.5 to 5 d. These theoretical values of core

rotational velocity, based upon simple principles of conservation of angular momentum, energy and torque flow from the Sun, give a total oblateness of 3.4×10^{-5} for the photosphere which is in close agreement with the observed value of 4.5×10^{-5} .

Three interesting problems still remain. How does the solar core maintain its rapid rotation, bearing in mind that it is a highly conducting plasma? Also what effect does this oblateness have on celestial mechanics? The value obtained would produce a 4.05 s of arc per century advance in the perihelion of Mercury. This opens up the question of the accuracy of the general relativistic tensor theory of gravitation in comparison, say, with the scalar tensor theory. Third, can the rapid rotation of the core produce a lower central temperature in the Sun and thus help explain why the neutrino flux is less than expected?

It must be mentioned, however, that not everyone agrees with the oblateness values obtained by Dicke and Goldenberg. Hill and Stebbins in a paper scheduled for the September edition of *The Astrophysical Journal* discuss observations they have made at the University of Arizona's observatory in the Santa Catalina Mountains. The value they obtained for the oblateness is $(8.6 \pm 5.9) \times 10^{-6}$ which indicates that the Sun's shape is indistinguishable from that of a sphere. □

Nitrogen in synthetic diamond

from John Walker

DIAMONDS might be a girl's best friend, but in many respects they are very puzzling to scientists. One of the most intriguing problems concerns nitrogen, which is a very common impurity in diamond, and which has recently been studied in synthetic crystals by some Soviet scientists (Yu. A. Klyuev, V. I. Nepsha, and A. M. Naletov, *Sov. Phys. Solid State*, **16**, 2118; 1975). Nitrogen occurs in aggregated form in most natural diamonds, but in a few rare crystals the nitrogen atoms are isolated, each one replacing a single carbon atom. These crystals can be detected because the nitrogen is paramagnetic—it can be observed in magnetic resonance experiments.

Surprisingly enough, synthetic diamonds usually contain isolated paramagnetic nitrogen rather than clusters. To investigate this difference, and to check the earlier magnetic resonance work on natural diamond, Klyuev *et al.* have grown synthetic diamonds doped with the rare nitrogen isotope ^{15}N . This isotope has a nuclear spin of one-half, which gives rise to a magnetic

HLA histocompatibility system

In the article "Histocompatibility testing international" which appeared in the News and Views Section last week, the designation of the HLA system was changed during editing to HL-A. The WHO nomenclature committee mentioned in the report did in fact recommend that the whole region should now be designated HLA.

resonance spectrum somewhat different from the ^{14}N which predominates in natural diamond and has a spin of unity. The Russian workers were able to confirm the earlier work, allowing for the different nuclear spin.

Nitrogen in diamond can also be detected using infrared spectroscopy. The absorption spectra of the ^{15}N diamonds were very similar to those of ^{14}N crystals, except that a peak at $1,135 \text{ cm}^{-1}$ in the latter had shifted to $1,120 \text{ cm}^{-1}$. This was accounted for by the different masses of the two isotopes. A second peak, at $1,282 \text{ cm}^{-1}$, which can be caused by nitrogen clusters, was relatively strong in the doped crystals, irrespective of the isotope used. Undoped diamonds were also found to contain paramagnetic nitrogen, presumably as a result of contamination from the atmosphere, but had a relatively weak $1,282 \text{ cm}^{-1}$ peak. This suggests that nitrogen clusters are present as a minor species in doped synthetic crystals.

The most likely cause of this difference in nitrogen's behaviour in natural and synthetic diamonds is that different pressures and temperatures are used in the two crystallisation processes. Laboratory studies by the same authors (Yu. A. Klyuev, Yu. A. Dudenkov, and V. I. Nepsha, *Geo. chem. Int.*, **7**, 781; 1973) have shown that relatively high pressures and low temperatures during the synthesis process create cubic crystals which contain a lot of dispersed nitrogen, but few aggregates. Lower pressures and higher temperatures create octahedral crystals with a lot of nitrogen clusters, very similar to natural diamonds. What is not clear is whether the aggregates are formed directly during the crystallisation process, or whether they grow afterwards, in the high pressures and temperatures needed for synthesis. The authors favour the latter hypothesis, but further work is needed to decide the issue. The fact that synthetic diamond can now be doped with a specific isotope indicates how well the process can be controlled. This has implications not only for diamond physicists, but also in the realm of new applications. □

review article

Electrons in glass

Nevill Mott*

Our understanding of the behaviour of electrons in glasses, liquids and non-crystalline materials generally is decades behind the detailed theory available for crystals such as silicon and germanium. This article describes the advances made in the past fifteen years. It includes the work of the Leningrad school on amorphous semiconductors, the reason why they cannot usually be doped, the concept of "variable-range" hopping and the Cohen-Fritzsche-Ovshinsky and other models for the conduction band, and the reason why glass can be transparent. A crucial experiment, that of two-dimensional conduction in an inversion layer at a Si/SiO₂ interface, is described. Particular emphasis is given to concepts about which there is a disagreement and indeed controversy, such as the "mobility edge", the "minimum metallic conductivity" and the interpretation to be given to the ovonic threshold switch.

EXTRACTION of metals and the manufacturing of glass are two of our oldest technologies; metals are important because they are strong and ductile and glass because it is transparent. And yet it is remarkable that a serious attempt to understand the ductility of metals in terms of the movement of atoms came later than, for instance, the discovery of the neutron, though now it is fully achieved; on the other hand the transparency of glass and equally the properties of some glasses as semiconductors are still not properly understood, and cannot yet be calculated in the detailed way that the application of quantum mechanics has made possible for crystalline materials such as silicon. At the same time there has in the past few years been a surge of interest in the electrical properties of glasses and of non-crystalline—that is to say amorphous—materials generally; this article describes what has been happening, and why.

Interest arose from several sources. The photocopying process known as xerography makes use of photoconduction in amorphous selenium and led to very extensive work on that material in the Rochester Laboratories of the Xerox Corporation. The development by Ovshinsky and coworkers of an electronic switch based on thin glassy films of a mixture of selenium, tellurium, arsenic, germanium (STAG glasses) started a lively controversy which is not yet over. Work in Leningrad, Prague and Bucharest pioneered some aspects of the subject; in particular Kolomiets (for review see ref. 1) in the Ioffe Institute at Leningrad showed that the chalcogenide (that is, based on S, Se, Te) glasses, such as As₂Te₃ and the STAG glasses, behaved like intrinsic semiconductors and with a resistivity which, markedly unlike crystals, could not be much lowered by doping.

On the theoretical side the work of Ziman² showed that electrical conduction in one class of non-crystalline material, liquid metals, could be very simply understood. Also in 1958 P. W. Anderson's difficult paper³ on the absence of diffusion in certain random lattices, which he himself says is "often quoted but rarely read", introduced the idea of localisation: whereas an extra electron introduced into a crystal, with energy (in the jargon of the subject) "in the conduction band", can move quite freely but may be trapped by impurities, the mere absence of crystalline order can lead to a range of energies for which

an electron is trapped even in the absence of impurities. Anderson's paper proved controversial and—like many new ideas—took some ten years and many international conferences to establish itself.

With this introduction I shall return to the fact that glass is transparent. In crystalline materials our understanding of the difference between transparent non-metals and opaque metals goes back to the first application of quantum mechanics to solids by Bloch, Peierls and Wilson in the early 1930s. They treated electrons as freely moving, and described by wave functions with well defined wavelengths; for certain wavelengths Bragg reflection from the lattice takes place, leading to the well-known energy gaps and to the concept of full and empty bands for the allowed energies of the electrons. A transparent non-metal is a substance in which a full band is separated from an empty band by an "energy gap", with energy greater than the quantum of visible light. But this description depends essentially on the crystallinity of the material; there are no sharp Bragg reflections from glasses for electrons, any more than there are for X rays. In fact, when theorists round about 1968 first noticed that there was a problem here, one paper at an international conference was entitled "How can there be an amorphous semiconductor?" One could ask too "How can there be a transparent glass?", and this is part of the same question.

A first clue came from the discovery by Kolomiets¹ that, as already stated the low conductivity of the chalcogenide glasses is not increased even by very strong doping. The same is true of amorphous films of silicon which can be deposited by various methods. When the film recrystallises, but not before, the impurities will act as donors and will cause the conductivity to shoot up. The most likely explanation is the following⁴. In crystalline silicon, which has the diamond structure with each of the four valence electrons taken up in a covalent bond, an atom of phosphorus (with five electrons) is incorporated substitutionally, four electrons being used in bonds and the fifth being very weakly bound, so that at room temperature it is free to move and can contribute to a current. In a glass or in amorphous silicon, on the other hand, the structure will normally accommodate itself so that any atom has the right number of neighbours to accommodate all the electrons in bonds; phosphorus if pentavalent should have five, or if trivalent three. The absence of freely or loosely bound mobile electrons is why any

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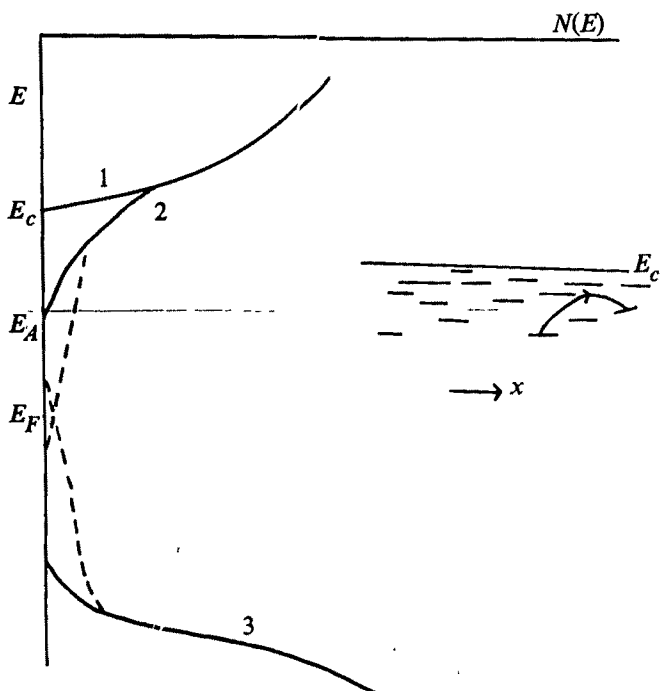


Fig. 1 Density of states $N(E)$ in an amorphous semiconductor plotted against energy. 1, Shows the form of $N(E)$ for the conduction band of a crystalline semiconductor; 2, the probable true form for the amorphous material; 3, the valence band and the dotted lines the "tails" as proposed by Cohen, Fritzsche and Ovshinsky. E_F is the Fermi energy and E_c the mobility edge. On the right the horizontal lines show energy levels in the traps (localised states), x denoting the position on space occupied by the traps. The line with the arrow shows a typical hopping process.

glass, is transparent. Colour in stained glasses is due to transition metal ions, where the inner electrons do not necessarily form covalent bonds and therefore are available for absorbing or scattering visible light.

A description of this kind is a long way from that usual among physicists for, say, crystalline SiO_2 or silicon, where all electrons are treated as free and the "energy gap" is a consequence of Bragg reflection. To treat the electrons as being stuck in bonds and to go no further seems a reversion to pre-quantum mechanical thought⁵. At present very active endeavours are in progress to bring a proper theoretical treatment to this model. In spite of a great deal of success, this work has not yet answered what is probably the most important question for glassy semiconductors, namely, what is the density of electronic states near the bottom of the conduction band? This is where electrons with thermal energies will be. For a crystal this density behaves like \sqrt{E} , where E is the energy; in a glass there should undoubtedly be a "tail", due to disorder, as suggested in Fig. 1. Those who feel that a gap is inconsistent with a non-crystalline structure have thought that the tail should extend an indefinite distance into the gap (the dotted line in Fig. 1) and should overlap a tail from the valence band; this was indeed proposed by Cohen, Fritzsche and Ovshinsky⁶ (the CFO model). But it now seems almost certain from observations that the "tails" are for all practical purposes limited; unless the glass has structural defects such as missing atoms, the band has a lower edge (E_A in Fig. 1). The states in the tail (shown shaded) are localised in the sense of Anderson; that is to say they consist of traps with a continuous range of energies. A sharp value of the energy E_c separating localised from non-localised states, is predicted and is known as the "mobility edge". Electrons with energies below E_c move from one localised state to another by the process known as "thermally activated hopping"—each time an electron moves it has to receive energy from lattice vibrations and its mobility tends to zero with the temperature; an

electron above E_c can move as in a crystal without help from lattice vibrations or thermal energy, though with a mean free path often as small as the interatomic distance.

Evidence for the existence of a mobility edge comes from experiments on the drift mobility of electrons injected into various materials, particularly experiments by Spear⁷ and coworkers on silicon glow-deposited from silane. These, unlike most evaporated or sputtered films, do seem to fall on microscopic voids, the presence of which makes the application of the theory for a continuous medium open to doubt. But perhaps another class of phenomenon gives a clearer demonstration of the concept. These are phenomena in which in a solid or liquid there is a degenerate gas or free electrons, so that the material behaves in all respects like a metal, but in which, as in a liquid metal, the positions of the atoms lead to a random electric field.

In liquid metals the random field leads to random scattering of the electrons, and as in Ziman's theory⁸ the scattering determines the mean free path. But—as is shown in Fig. 1, in the tail of a band the states are localised, and the electrical properties depend on whether the Fermi energy E_F , that is the energy of the highest occupied state, lies below E_c in the localised region, or above E_c . The conductivity of a metallic system depends only on the electrons with energies near the Fermi energy E_F , and so if E_F lies below E_c conduction is by hopping. I first showed that the conductivity as a function of temperature will then follow the law⁸

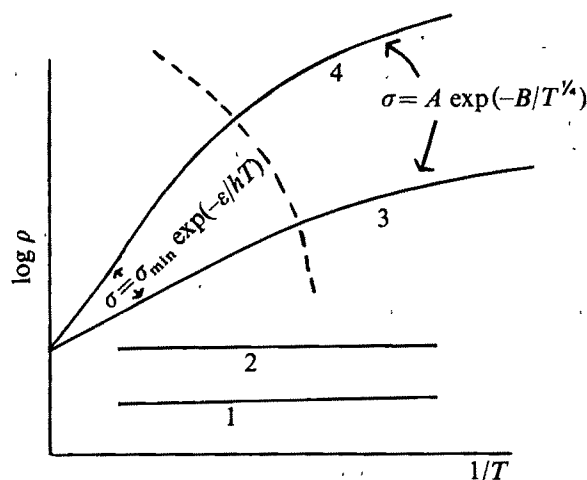
$$\sigma = A \exp(-B/T^{1/4}) \quad (1)$$

(where $T^{1/4}$ becomes $T^{1/3}$ in a two-dimensional system), a law which has been discussed extensively to find out in what conditions it should be valid. If on the other hand E_F lies above E_c , the conductivity tends to a finite value as the temperature tends to zero, which we can call metallic behaviour. If one then can vary the Fermi energy, for instance by varying the composition or the degree of disorder, or both, a kind of metal-insulator transition will occur, which has been named an "Anderson transition". The type of resistivity-temperature behaviour expected is shown in Fig. 2. When E_F lies below E_c , the conductivity σ at low temperature will follow the law (1); while at high temperatures current will be carried by electrons excited to E_c , so

$$\sigma = \sigma_{\min} \exp(-\varepsilon/kT), \quad \varepsilon = E_c - E_F$$

σ_{\min} , the lowest value of the conductivity for which there is no activation, is essentially that which one expects when the scattering is so strong that the mean free path becomes comparable

Fig. 2 Behaviour of the resistivity ρ of a substance in which there is a degenerate electron gas of which the Fermi energy can be shifted through the mobility edge. Disorder is increasing at E_F in the sequence numbered 1, 2, 3, 4.



with the distance between atoms (a). It is about $0.1e^2/\hbar a$, or in the neighbourhood of $1,000 \Omega^{-1} \text{ cm}^{-1}$. I have designated this quantity the "minimum metallic conductivity"⁹; the concept is not accepted by all theorists¹⁰ and there is a lively controversy at present on whether, as I believe, such a quantity exists.

There are many systems in which an Anderson transition can be observed, usually by using a series of specimens of varying composition or in heavily doped magnetic semiconductors by varying a magnetic field. But one of the neatest is the investigation due to Pepper^{11,12} and coworkers on conduction at the interface between n- or p-type silicon and a layer of silicon dioxide grown on the surface, using for instance the MOSFET (metal oxide silicon field effect transistor). In this device, by applying a voltage (the gate voltage) across the oxide layer, the number of electrons at the interface can be varied at will. Moreover these form a truly two-dimensional gas, and at helium temperatures the gas is degenerate. Also the oxide formed on the silicon contains positive and negative charges, distributed at random; therefore the electrostatic field seen by the electrons will vary in a random way, so that a "tail" is formed to the density of states, as in Fig. 1. By varying the gate voltage, therefore, and measuring the resistivity in the plane of the interface, it has proved possible to reproduce the behaviour of Fig. 2 in some detail. The experimental work, carried out in the Cavendish Laboratory in collaboration with Plessey Ltd, has two major achievements. Over four orders of magnitude the conductivity below 3 K has been found to be of the form

$$\sigma = A \exp(-B/T^n) \quad (2)$$

with $n = 0.32 \pm 0.02$, a measurement which established the two-dimensional nature of the phenomenon, which in all respects follows the predicted behaviour of Fig. 2; and secondly the minimum metallic conductivity can be established experimentally.

For a two-dimensional system the calculated value is about $0.1e^2/\hbar$, and it has been suggested by Thouless (unpublished) that this is a universal constant, not depending on the form of disorder; in conventional units this is about $10^{-5} \Omega^{-1}$.

The metal-insulator transition shown in Fig. 2 is essentially due to disorder and is quite different from the "Mott transition"^{13,14} which is first order and a consequence of interaction between electrons.

Conductivity having the forms (1) and (2) is known as variable-range hopping. It arises because the probability that an electron jumps from one localised state to another at a distance R is of the form.

$$C \exp(-2\alpha R - (W/kT)) \quad (3)$$

where W is the energy difference between them and α is a constant. The greater R the greater the choice of state, so if the electron chooses the smallest available value of W ,

$$W \propto [1/(4\pi/3)] R^3 N(E_F)$$

Equation (1) comes from choosing R to maximise the quantity in equation (3). In two dimensions πR^2 replaces $(4\pi/3)R^3$, so equation (2) follows. Variable-range hopping has been extensively observed, but deviations from equation (1) occur in many cases, probably because the assumption made that C and $N(E)$ are sufficiently independent of energy and temperature may not be valid.

Experiments such as that described above give us a lot of confidence in the concept of a mobility edge in the conduction band of any disordered system, and in the possibility of applying it to real glassy materials, and indeed there is a multitude of papers in which this is done (see for instance ref. 13). One of the difficulties many theorists had in accepting Anderson's localisation idea, together with a continuous distribution of trap energies, was the intuitive feeling that an electron in a trap, if it tunnelled far enough, would find another trap with exactly the

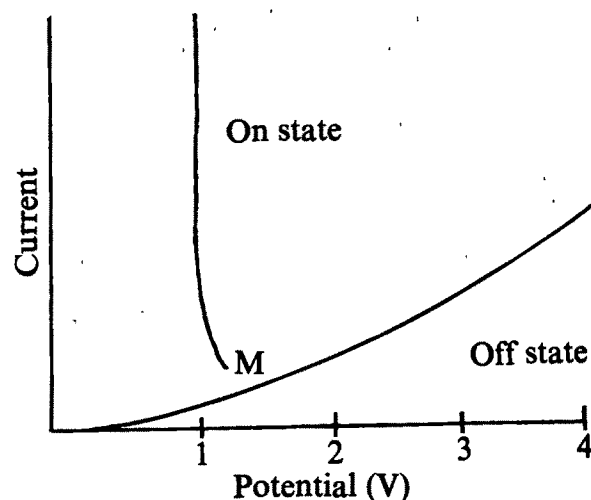


Fig. 3 Current-voltage curve for an ovonic threshold switch in on- and off-states.

same energy, and so the conductivity could not be temperature-activated and should not tend to zero at low temperatures. The reason why this is not so is rather subtle⁴; the experimental evidence from results such as those of Fig. 2 is, however, overwhelming. Also the measurements by Spear and coworkers⁷ of the drift mobility of injected electrons, that is to say the rate, at which they drift across a specimen under the influence of an electric field, gives very firm evidence; at low temperatures the electron hops from one localised trap to another, at high temperatures it is excited to the mobility edge. But in many materials, particularly chalcogenides the drift mobility seems to depend on structural defects, and indeed we have little idea of their nature or whether it is even in principle possible to have a fully coordinated glass without them.

I feel that some of the most important developments in this field are likely to concern the kinds of structural defects in glasses, their effect on photoconduction and their relation to recombination centres in crystals. Even in crystals the mechanism ("multiphonon transitions") by which electrons and holes recombine without emission of radiation is imperfectly understood, in spite of much theoretical work; in silica glasses, we have very little idea. A challenging and controversial paper by Anderson¹⁴ may be relevant here, concerned with the way in which an electron in a trap deforms its surroundings. Also amorphous silica in silicon technology and amorphous tantalum oxide in anodic capacitors present a host of problems.

There is another problem which obstinately defies complete understanding, namely the mode of action of the threshold switch developed by S. R. Ovshinsky¹⁵ in his firm Energy Conversion Devices. In its simplest form this consists of a layer of STAG glass about $1 \mu\text{m}$ thick between two molybdenum electrodes. Figure 3 shows schematically how the switch behaves. In the off-state the material is a semiconductor with highly temperature-dependent impedance; in the on-state, which can be maintained as long as the current does not fall below M (the minimum holding current), the current is quite insensitive to temperature. It can reach more than 10 mA, probably in a channel a few μm thick. The controversy is about whether the on-state is due to a thermal instability, a hot channel at perhaps 500°C being formed¹⁶, or whether the channel is merely warm and electrons and holes are injected from the two electrodes¹⁷. A measurement of the temperature of the conduction channel would resolve the problem, but this has not proved possible; the radiation from a switch in the on-state has, however, been observed^{18,19} and bears no resemblance to black body radiation.

The controversy has been heated and at times bad-tempered, because the threshold switch was presented with commercial claims as something new, and some scientists in other industrial

laboratories claimed that it was a thermal instability and they had known about it for a long time. In spite of the many successes of the thermal theory I believe, though without absolute certainty, that there is indeed something new here, for which Ovshinsky and his many collaborators in American universities deserve credit. If electrons and holes are injected, degenerate gases of electrons and holes must be formed and the Fermi energies of one or other of them must lie above the corresponding mobility edge; otherwise the large observed currents with no thermal activation could not occur.

Indeed, just as this article is going to press, a good deal of new evidence is appearing in favour of an electronic and against a thermal model (ref. 20 and E. Peterson, G. W. Lake and D. Adler, unpublished).

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articles

How specific are nuclear “receptors” for thyroid hormones?

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The presence of “high-affinity-saturable” binding sites for thyroid hormones of similar characteristics not only in isolated nuclei but in all the major extranuclear cellular components, as well as the failure of cytosol to promote nuclear binding, invalidates the analogy with steroid hormone receptors and necessitates a more critical assessment of the physiological relevance of current approaches to binding of thyroid hormone in vitro to nuclear preparations.

THE identification of specific hormone-binding components or “receptors” in target cells is considered to be a primary requirement for an understanding of hormone action. It is now commonly held that polypeptide hormones exert their action by interacting with binding components on the surface of the cell¹, whereas some, but not all, small molecular weight hormones act through intracellular receptors². The latter generalisation is based largely on the concept of a two-step process of steroid hormone action whereby the hormone would first interact with a cytoplasmic receptor after which the hormone-receptor complex would be transferred into the nucleus where the complex or the hormone alone would initiate its physiological action^{2–6}. Since steroid^{2,5–8} and thyroid hormones^{9,10}, in common with other growth and developmental hormones^{11,12}, markedly stimulate nuclear transcriptional activity, the concept of a nuclear site of specific hormone receptor(s) has recently been extended to thyroid hormones^{13–23}.

Early studies with cellular extracts revealed that both the “soluble” cytoplasmic fraction (“cytosol”) and various particulate subcellular fractions (mitochondria, microsomes, nuclei, debris, and so on) were equally effective in binding radioactive thyroid hormones *in vivo* or *in vitro*^{24–28}. These

studies were performed with hormones of low specific radioactivity so that they could have only indicated nonspecific binding sites and not detected the easily saturable and physiologically more relevant binding sites or “receptors”. Recent studies on nuclear location of thyroid hormone receptors have been based on the use of ¹²⁵I-labelled tri-iodothyronine (T₃) of high specific radioactivity and these suggest that a rat liver nucleus has about 5 × 10³–10 × 10³ “specific” sites for this hormone^{15,17,18,20}. Before these sites can be considered physiologically significant, however, it is essential to demonstrate that other subcellular components do not comprise sites of similar T₃-binding characteristics. In view of the importance of this question it may seem surprising that no studies have so far examined, in identical conditions, the thyroid hormone-binding characteristics of the major subcellular fractions derived from the same tissue, although the cytosol fraction is known to account for a large fraction of both high and low affinity sites in different tissues^{24–32}.

I shall describe here experiments which show that the binding of highly radioactive T₃ *in vitro*, as used in other recent studies, is not restricted to the nucleus or nuclear extracts, but that preparations of cytosol, plasma membrane, microsomes and inner mitochondrial membranes all exhibit similar “high-affinity” binding characteristics. The studies described below only deal with rat liver for the sake of comparison since most recent work on nuclear receptors is restricted to this tissue. All subcellular preparations exhibited two major classes of T₃-binding sites: (1) those with high affinity and relatively low capacity (commonly termed “specific”), and (2) those with low affinity and virtually non-saturable (“nonspecific”). This paper also describes a direct method for determining free and bound thyroid hormone on DEAE-cellulose filter disks, adapted from a procedure described earlier for glucocorticoids³³.

Characteristics of binding

Adsorption of the ^{125}I - T_3 -protein complex on DEAE-cellulose gave a linear binding response for both particulate and soluble cellular components (Fig. 1). That the fraction of radioactivity retained on DEAE-cellulose filters represents T_3 bound to macromolecules was confirmed by precipitation and washing with trichloroacetic acid of identical samples. By treating the solution of radioactive T_3 with DEAE-cellulose just before use, the fraction of ^{125}I retained on "blanks" was reduced to below 4%. Any ^{125}I -iodide would thus be retained by the filters but not free T_3 or iodothyronine analogues. A major advantage of this procedure over those commonly in use is that the radioactivity measured on the adsorbent is a direct measure of the T_3 bound to protein or nucleic acids, whether the latter are in solution or not, and, therefore, more accurate than indirect methods involving the introduction of an

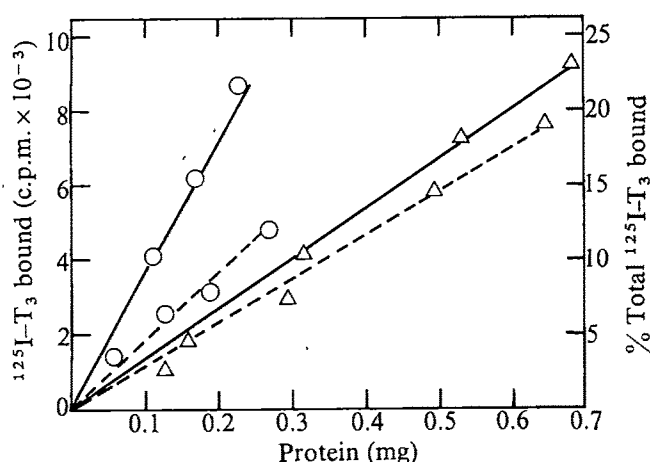


Fig. 1 Binding of ^{125}I -tri-iodo-L-thyronine (Abbott Laboratories, specific activity 322 Ci mmol^{-1}) to increasing amounts of nuclei and cytosol from livers of normal and thyroidectomised rats (150 g, Carworth Europe). Nuclei were freshly prepared from three livers by the method of Blobel and Potter³⁴, except that the homogenising medium was 0.25 M sucrose, 50 mM Tris-HCl (pH 7.6) 12.5 mM NaCl, 12.5 mM KCl and 5 mM MgCl_2 (STKNaM) and the nuclear pellet suspended in the same medium at a concentration of 4–6 mg DNA ml^{-1} . Cytosol was prepared from rat liver, perfused with 100 ml of Krebs-Ringer phosphate buffer, washed and homogenised in 2 vol of STKNaM. After two centrifugations at 10,000g for 20 min, the mitochondria-free supernatant was centrifuged at 105,000g for 3.5 h. The resulting supernatant, termed "cytosol", was stored at -30°C in small aliquots until use. Because of hormone adsorption problems, contact with glassware was avoided for all binding studies which were carried out in polycarbonate tubes, while all volumetric measurements were performed with polypropylene pipettes and syringes. Tissue preparations were incubated for 10 min at 37°C in a total volume of 260 μl made up with STKNaM. ^{125}I - T_3 was diluted with STKNaM to reduce the propylene glycol concentration down to below 5%. In this experiment, all samples were incubated with ^{125}I - T_3 at a final concentration of 2.5×10^{-10} M (43,000 c.p.m.). In this and all other experiments, the diluted ^{125}I - T_3 solutions were passed through three layers of DEAE-cellulose filter disks (Whatman DE 81), presoaked in STKNaM, through a Millipore plastic Swinney adaptor attached to a 1-ml syringe just before incubation. This procedure removed ^{125}I -iodide and ensured low blanks. At the end of the incubation, the contents of the tube were immediately deposited on two layers of DEAE-cellulose disks, presoaked in 20 mM Tris (pH 7.6) and 1.5 mM EDTA held in a Millipore filter manifold. The vacuum was adjusted so that filtration took 10–20 s only and the contents of the tubes and filters were rinsed 5–7 times with 1 ml of Tris-EDTA solution. The filters, before they were dried, were crushed into polycarbonate tubes and ^{125}I determined in a Packard Auto-gamma counter. In all experiments, protein-free controls were processed simultaneously and corrections were made for blanks which were usually of the order of 2–4% of the total input radioactivity. Protein, RNA and DNA were measured by standard procedures^{35–38}. Each value is the mean of four determinations on two parallel incubations. The average variability was $\pm 6\%$. \circ , Nuclei; Δ , cytosol; — — —, normal; — — —, thyroidectomised.

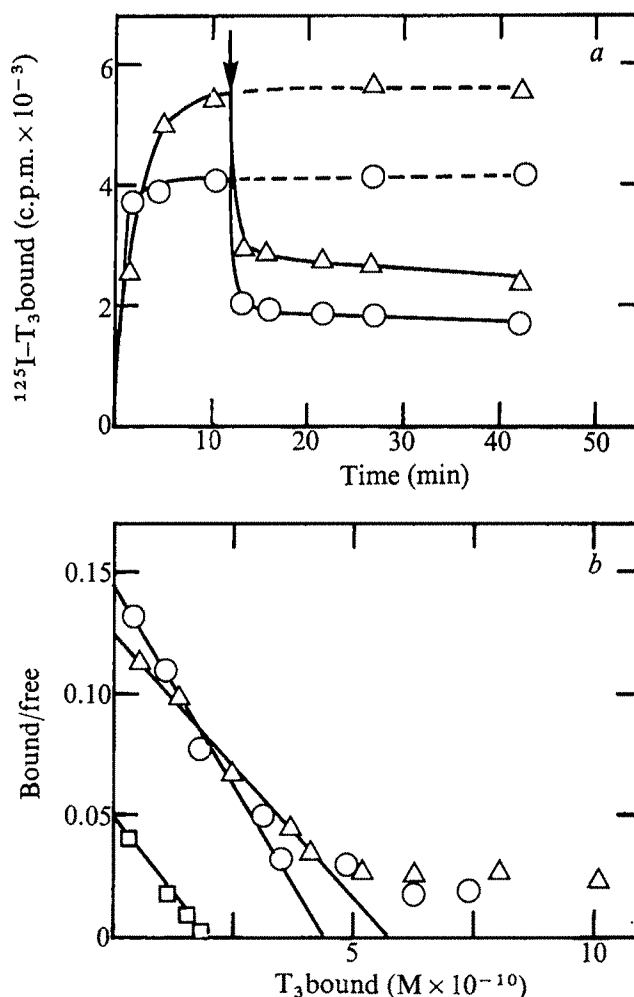


Fig. 2 T_3 -binding characteristics of nuclei, nuclear sap and cytosol from thyroidectomised rat liver. Nuclei and cytosol were prepared as described in Fig. 1 and nuclear sap as described in legend to Table 2. *a*, Time course and reversibility of binding. Cytosol (1.1 mg protein) and nuclei (190 μg protein) were incubated with 100 μg of ^{125}I - T_3 (36,000 c.p.m.) at 37°C for different periods of time. To one set of tubes, 6 μg of non-radioactive T_3 were added at 12 min (indicated by the arrow) and the incubation continued for a further 30 min. To the other set no "cold" T_3 was added. The radioactive T_3 bound to cytosol or nuclei was determined as in Fig. 1. \circ , Nuclei; Δ , cytosol; \square , nuclear sap. *b*, Scatchard plot analysis of affinity and capacity of binding of ^{125}I - T_3 to nuclei, cytosol, and nuclear sap. Different amounts of T_3 , containing a fixed amount of ^{125}I - T_3 (35,000 c.p.m.) were incubated with nuclei (175 μg protein), nuclear sap (45 μg protein) and cytosol (236 μg protein) for 10 min at 2°C . The other procedures are as described in Fig. 1. \circ , Nuclei; Δ , cytosol; \square , nuclear sap.

insoluble "binder" of free hormone, such as dextran-coated charcoal.

All the physicochemical measurements in this work were made in parallel in normal and thyroidectomised animals since nearly half the liver nuclear T_3 -binding sites measured in thyroidectomised rats are occupied by endogenous thyroid hormone in normal animals^{17,20,38}. The binding of ^{125}I - T_3 at low concentrations (2×10^{-10} – 5×10^{-10} M) was very rapid ($T_{1/2}$ of 1–3 min) for both nuclei and cytosol alone (Fig. 2*a*). Displacement of radioactive hormone bound to these fractions by the addition of a large excess of unlabelled T_3 gave, at equilibrium, an approximate dissociation half time of 70 min for cytosol and 36 min for nuclei. ^{125}I - T_3 was also similarly displaced by higher concentrations of L-thyroxine and other iodothyronine analogues (not shown in Fig. 2*a*). When the binding of T_3 was measured at different hormone concentrations, it was found that for all cellular components from thyroidectomised liver, there exist at least two major classes of

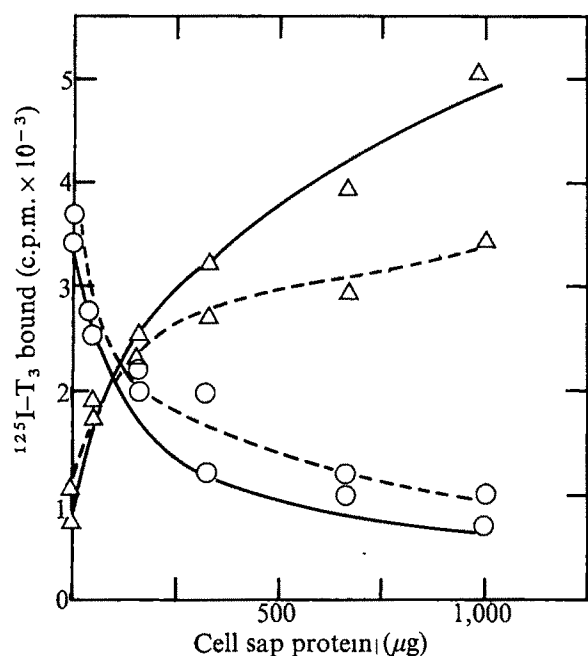


Fig. 3 Competition between cytosol and nuclei for the binding of $^{125}\text{I-T}_3$ at low and high concentrations of the hormone. A fixed amount of nuclei (180 μg protein) were incubated with different amounts of cytosol at 37°C for 10 min with either $4 \times 10^{-10}\text{ M}$ (17,000 c.p.m.) or $2 \times 10^{-7}\text{ M}$ (25,000 c.p.m.) $^{125}\text{I-T}_3$. At the end of the incubation, the nuclei were separated from the cytosol by centrifugation at $2,000g$ for 5 min and the bound radioactivity determined in each component by adsorption to DEAE-cellulose disks. Values for "O" cell sap are from incubations carried out in the absence of cell sap and therefore represent bound $^{125}\text{I-T}_3$ in the nuclear supernatant. Other conditions as in Fig. 1. \circ , Nuclei; \triangle , cell sap; —, $4 \times 10^{-10}\text{ M T}_3$; ---, $4 \times 10^{-7}\text{ M T}_3$.

binding sites: those that have high affinity and low capacity and those that exhibit a 100-fold lower binding affinity but are difficult to saturate. Data, such as those presented as Scatchard plots in Fig. 2b, gave values for association constant (K_a) of $1.2 \pm 0.3 \times 10^8\text{ M}^{-1}$ and $0.8 \pm 0.3 \times 10^8\text{ M}^{-1}$ for nuclei and cytosol respectively. The binding capacity for the same two fractions was $3.0 \pm 0.9\text{ pmol}$ and $0.7 \pm 0.2\text{ pmol}$ per mg protein, respectively. These values are within the same range as those reported elsewhere for nuclei^{15,17,18,20} and cytosol²⁸⁻³⁰ *in vitro*, especially when one takes into account the quite different methods used for studying binding.

Nucleus and cytosol

An outstanding characteristic of intracellular translocation of steroid hormones is the process by which the hormone has to be complexed to cytosol receptors before it can move into the nucleus. The receptor in the cytosol is different from the "acceptor" sites in the nucleus and the translocation of the hormone from one subcellular site to the other requires a temperature-sensitive activation step^{2-8,39}. In order to test the validity of a steroid hormone analogy for thyroid hormone

receptors, it was reasoned that if the same principle governed the two classes of hormones, then coincubating $^{125}\text{I-T}_3$ with nuclei in the presence of cytosol should enhance in a cooperative fashion the concentration of the hormone in the nucleus. On the other hand, an independent interaction between the hormone and the two subcellular fractions should lead to a competition between the subcellular components.

Figure 3 shows that the addition of increasing amounts of cytosol to nuclei led to a corresponding decrease in the radioactivity recovered with the nuclei. When one takes into account the binding constants and capacities given in Fig. 2b (see also Table 2) for the two fractions, the results can be accounted for by competition by the two subcellular fractions for the available T_3 , governed by simple mass action kinetics. Similar results were obtained at both low ($4 \times 10^{-10}\text{ M}$) and high ($2 \times 10^{-7}\text{ M}$) concentrations of the hormone, that is at concentrations above and below saturation of binding sites of high affinity and limited capacity. The results in Fig. 3 were obtained at 37°C so that a cytosol receptor activation step, analogous to that involving steroid hormones, should have given the opposite result. From Table 1 it is clear that preincubating cytosol at 37°C failed to enhance the uptake of the hormone into nuclei at either 2 or 37°C . Many permutations, to be reported elsewhere, of coincubating nuclei and cytosol at different temperatures also failed to reveal a temperature-dependent activation step for a cytosol-nuclear transfer mechanism. Although they did not specifically look for a thermosensitive activation step, Surks *et al.*¹⁷ and De Groot and Torresani¹⁸ also concluded that the cytosol did not play a role in the transfer of the hormone to the nucleus. The steroid hormone analogy for early events in hormone action is clearly not applicable to thyroid hormones.

Other cellular components

The above findings of not too dissimilar classes of competing binding sites in the nucleus and soluble cytoplasm raised the question of their specificity with respect to other cellular components. An examination of all the major subcellular and subnuclear fractions was undertaken in which the physicochemical characteristics for $^{125}\text{I-T}_3$ binding were measured simultaneously and under identical conditions. Very rigorous conditions were established in the preparation of subcellular fractions to eliminate mutual contamination. Thus, a subfraction of plasma membranes comprising only the outer face of the cell surface⁴¹ was used in order to exclude microsomal contamination. Since the outer membrane of mitochondria shares some common components with the endoplasmic reticulum⁴², a preparation of only the inner mitochondrial membrane⁴³ was used. Similarly, nuclei were washed with an amount of detergent (0.2% Triton) known to remove both the outer and inner nuclear membranes⁴⁴, again in order to eliminate microsomal contamination. Recently, many reports have indicated that the nuclear site for thyroid hormone binding is in the chromatin from which it can be extracted with 0.4 M KCl ^{14,17,20-22}. Such treatment of chromatin is known to extract the bulk of non-histone acidic nuclear proteins⁴⁵ as well as acceptor sites for oestrogen, progesterone, androgen and corticosteroids in their respective target nuclei^{2,4}.

Table 1 Absence of temperature-dependent activation step for transfer of bound $^{125}\text{I-T}_3$ from cytosol to nuclei

Temperature of preincubation of cytosol + $^{125}\text{I-T}_3$ ($^\circ\text{C}$)	Temperature of incubation of cytosol + nuclei ($^\circ\text{C}$)	Bound $^{125}\text{I-T}_3$ (c.p.m.) recovered in	
		Nuclei	Cytosol
2	2	1,923	2,422
2	37	1,793	2,479
37	2	2,026	1,941
37	37	1,701	2,294

Cytosol from thyroidectomised rat liver (1 mg protein in 0.16 ml of STKNaM) was preincubated with $2 \times 10^{-10}\text{ M }^{125}\text{I-T}_3$ (35,000 c.p.m.) for 10 min at either 2 or 37°C and then chilled in ice for 5 min. 0.1 ml of a freshly prepared nuclear suspension (275 μg protein) was then added and the mixture incubated for 10 min at 2 or 37°C after which nuclei and cytosol were separated as indicated in Fig. 3. The fraction of $^{125}\text{I-T}_3$ bound was determined as described in Fig. 1.

Table 2 Characteristics of binding of ^{125}I -tri-iodothyronine to nuclear and extranuclear components

Component	Rat	Association constant K_a (M^{-1})	No. of binding sites M (pmol per mg protein)	$T_{1/2}$ (min) for Association	$T_{1/2}$ (min) for Dissociation
Nuclei	Normal	2.4×10^8	1.73		
	Thyrex	1.3×10^8	2.96	2.9	36
Cytosol	Normal	8.0×10^7	0.67		
	Thyrex	2.3×10^8	0.69	3.0	70
Nuclear sap	Thyrex	1.7×10^8	2.10	5.9	
0.4 M KCl Extract of chromatin	Thyrex	9.3×10^7 *	0.15	8.5	
Inner mitochondrial membranes	Normal	7.3×10^7	0.27	7.5	20
Plasma membranes	Normal	8.2×10^8	0.32		
Microsomes	Thyrex	5.9×10^8	0.08	8.0	80

Nuclei and cytosol were prepared as described in Fig. 1. Nuclear sap was prepared by gentle sonication of nuclei according to the method of Tata and Baker⁴⁶. The chromatin pellet remaining after removal of nuclear sap was extracted twice at 2 °C with 0.4 M KCl (chromatin from 3 g equivalent liver ml^{-1}) and both the 0.4 M KCl extract and nuclear sap were dialysed overnight against three changes of 200 vol of STKNaM. Much of the protein in 0.4 M KCl extract precipitated out during dialysis and it was redissolved in 0.4 M KCl. Both the nuclear sap and the salt extract of chromatin were concentrated in an Amicon (Minicon B15) dialysis concentrator to a protein concentration of 1–2 mg ml^{-1} . Plasma membranes were subfractionated to yield the outer face components according to Wisher and Evans⁴¹ and the inner mitochondrial membranes prepared by the method of Greenawalt⁴². Microsomes were the pellet obtained from the preparation used for obtaining cytosol. The binding characteristics were calculated according to the details given under Figs 1 and 2, except that the standard temperature was 37 °C. Nuclei, when not fractionated, were washed once with 0.2% Triton X-100 followed by two washes with STKNaM. Thyrex: thyroidectomised.

*The values for 0.4 M KCl extract of chromatin are rough estimates only because of the problem of protein precipitation in STKNaM.

Similar preparations were, therefore, also made from rat liver chromatin but after previous removal of "nuclear sap" which contains soluble nuclear proteins released by gently disrupting nuclei at isotonic salt concentration⁴⁶. Since some subcellular components of liver degrade thyroid hormone²⁸, comparison of thyroid hormone binding to different subcellular fractions was carried out both at 37 and 2 °C.

The results, summarised in Table 2, show that all subcellular fractions exhibited the presence of apparently "specific" and readily saturable T_3 -binding sites. Their relative affinities varied only over a range of ± 5 -fold, which is not considerable when considering that one is comparing binding of a ligand to macromolecules in solution for some components and in a suspension of membranes or complex macromolecular particles in others. The number of T_3 -binding sites per nucleus was of the order of 10,000 but the total binding capacity as expressed per mg protein or per g equivalent of tissue varied over a wide range for the different fractions. As regards fractionation of nuclei, in results to be presented elsewhere (J.R.T., K.M.B.S., and B.B., unpublished observations), more than half the ^{125}I - T_3 bound to nuclei was recovered in the euchromatin fraction. This is in agreement with recent reports from Baxter's laboratory^{23,40} but whether or not the endogenous hormone is directly bound to DNA as concluded by these workers or to non-histone protein in intact chromatin, as proposed by others^{14,15,20–22}, is difficult to decide. In our hands much difficulty was experienced in studying the binding under standard ionic conditions to the non-histone proteins extracted from chromatin by 0.4 M KCl because of precipitation of protein on lowering the salt concentration. On the other hand, the nuclear sap fraction, which represents soluble nuclear proteins released on gentle sonication of nuclei at relatively low salt concentrations⁴⁶, had T_3 -binding sites present at higher concentration (as expressed per mg protein) than in total euchromatin (J.R.T., *et al.*, unpublished). These findings (Table 2) of the ubiquitous distribution of T_3 -binding sites in nuclear and extranuclear fractions of rat liver are compared in Table 3 with the characteristics described by other workers for this and other tissues, thus establishing that such sites are certainly not restricted to the nucleus.

Hormone binding and action

The above results raise several questions concerning the relevance of binding of thyroid hormone to subcellular components *in vitro* to the initiation of the physiological actions of the hormone. First, do the T_3 -binding component(s) of K_a of 10^8 – 10^9 M^{-1} in the different subcellular fractions represent the same or different molecular species? An electrophoretic

or sedimentation analysis of the solubilised binding components of cytosol and nuclear sap would provide a partial clue. Whatever the answer, it seems that the emphasis given in recent work to nuclear binding of T_3 *in vitro* to explain thyroid hormone action is unjustified nor is the steroid hormone analogy valid. Second, the quantitative aspects of the binding raise some doubts about the relevance to physiological action. Except for the pituitary³⁸, only about 15–20% of the endogenous or exogenous hormone is associated with the nuclei from liver or kidney homogenates^{16,21,38}. The "specific" high-affinity binding of T_3 to various subcellular fractions reported here and elsewhere^{13–23,27–32,38} is not much different from that described in our earlier studies^{27,28} with hormone of low specific radioactivity binding mostly to the low-affinity, non-saturable sites. Moreover, both the high-affinity and low-affinity sites in the nucleus and elsewhere do not exhibit a stringent specificity with respect to the biological activity of thyroid hormone analogues. In fact, the relative affinities of various analogues reported for the easily saturable, high-affinity sites^{18,21,30,31} are very similar to those reported some years ago for the low-affinity, "nonspecific" sites^{27,28}. On the other hand, a high degree of correlation between physiological potency or anti-hormone activity has been a major argument in supporting the physiological relevance of nuclear binding of steroid hormones^{2,4,6–8,47,48}. The figure of 5–10,000 binding sites for T_3 per rat liver nucleus may also be too high if one considers the phenomenon in terms of specific induction of proteins through genetic activation. The affinity of the nucleus for tri-iodothyronine is not much greater than that exhibited by cytoplasmic fractions or of plasma thyroxine-binding globulin (TBG) for either tri-iodothyronine or thyroxine ($K_a = 2.0 \times 10^9 \text{ M}^{-1}$ and $2.4 \times 10^{10} \text{ M}^{-1}$, respectively)²⁸. Oppenheimer *et al.*¹⁶ calculated the affinity of the rat liver nucleus for T_3 *in vivo* as K_a of $4.7 \times 10^{11} \text{ M}^{-1}$, a value nearly 1,000 times higher than that reported from the same laboratory⁴⁷ for the "specific" interaction *in vitro*. Interestingly, intact lymphocytes bind T_3 with a K_a of $9.4 \times 10^{11} \text{ M}^{-1}$ (binding capacity = 6.5×10^{-4} pmol per 10^7 cells) but the location of the binding sites has not been described⁵⁰. The discrepancy between the relative affinities of nuclei *in vivo* and *in vitro* has been explained on the basis of the deleterious effects of ionic and other conditions *in vitro*¹⁷ but then the same argument should also apply to extranuclear fractions. There is, in fact, no direct evidence that the binding to nuclei *in vitro* tells us much about the properties of a possible true nuclear receptor for thyroid hormone that may be present in much lower concentration. Similar questions have been raised for extremely high-affinity nuclear binding for oestradiol present in amounts as low as

Table 3 Characteristics of binding of ^{125}I -tri-iodothyronine to "specific" high affinity sites in nuclear and cytosol preparations described by other workers

Preparation	K_a (M^{-1})	No. of sites	$T_{1/2}$ (min) for		Ref.
			Association	Dissociation	
Rat liver nuclei	6.1×10^8	2.0 pmol g^{-1} liver	3	14	15
	5.5×10^8	1.3 pmol g^{-1} liver			17
	2.0×10^9	0.8 pmol g^{-1} liver			18
Rat liver NHP*	1.6×10^9	6,000 per nucleus			22
Rat pituitary tumour cell line nuclei	2.9×10^8	5,000 per nucleus			19
Rat liver cytosol	4.3×10^7	0.53 pmol mg^{-1} protein			30
Rat kidney cytosol	2.5×10^7	2.9 pmol mg^{-1} protein			30
Dog liver cytosol	2.3×10^7	—			29
Pig pituitary cytosol	4.0×10^8	—	4	50	28

*NHP, Non-histone protein (nuclear proteins soluble in 0.4 M NaCl).

10–1,000 molecules per cell in the uterus^{6,51,52}. It is hard to visualise how physiologically important receptor molecules could be detected at that level for thyroid hormones by binding techniques currently available.

High-affinity steroid-binding sites have also been recently detected in extranuclear sites (plasma membranes, microsomes) in tissues where similar nuclear binding sites have been well characterised^{53–55}. An ubiquitous distribution of not only thyroid but also steroid hormone-binding sites leads to a consideration of possible multiple sites of initiation of actions of growth and developmental hormones. It is well known that multiple^{9–11,56} or "pleiotypic"⁵⁷ responses are elicited by all growth and developmental stimuli, whether they be hormonal or not, before induction of new protein or messenger RNA species. Many of these effects, such as the alteration of cell surface, permeability towards small molecules and ions, metabolism of cyclic nucleotides, and so on cannot be accounted for by an action exclusively initiated at the level of the cell nucleus. The generalisation that polypeptide hormones act at the cell surface whereas small molecular weight hormones act within the cell is a gross over-simplification, as far as the latter class of hormones are concerned; after all, most actions of catecholamines can be satisfactorily explained on the basis of their interaction with plasma membrane receptors associated with adenylate cyclase⁵⁸. One has, therefore, to consider the possibility that the final expression of a growth and developmental hormone may result from a cooperative action generated by simultaneous or sequential but independent interactions with more than one nuclear and extranuclear sites.

The above discussion does not diminish the role of the nucleus in the action of thyroid or steroid hormones nor of the importance of continuing with hormone-binding studies. Nor is it implied that receptors or acceptors of steroid hormones are of little consequence in hormone action. Indeed, increasing evidence is now gathering from genetic studies that the cytosol-nuclear translocation and the nuclear localisation of steroid hormone are intimately associated with their capacity to induce the synthesis of specific proteins^{59,60} and a similar analysis of responsiveness to thyroid hormones would help clarify some of the ambiguities raised in this paper concerning their binding to nuclear and extranuclear cellular components. What can be said is that thyroid hormones do not interact with their target cells in the same manner as do steroids and that the current emphasis on their nuclear site of action based on binding studies with tri-iodothyronine *in vitro* may not reveal a balanced picture of the location and distribution of true receptors and, therefore, the initial events leading to the physiological action of these hormones.

Detailed studies on the subcellular and subnuclear distribution of thyroid hormone binding sites will soon be published elsewhere.

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A major geothermal anomaly in the Gulf of California

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We have mapped a 3-km wide, high heat flow anomaly with a maximum value of $30 \mu\text{calorie cm}^{-2} \text{ s}^{-1}$ within a zone of seafloor extension in the central Gulf of California. From seismic reflection data and thermal modelling we suggest that the anomaly is caused by a 1-km wide basaltic intrusion which is roughly 100 m deep and less than 18,000 yr old.

SEAFLOOR spreading associated with the East Pacific Rise can be extended to inside the mouth of the Gulf of California, by correlating magnetic lineations¹. Further into the gulf the magnetic lineations are either disturbed by the thick sedimentary cover, or the lineations are weak or non-existent due to an intrusive or cooling mechanism that is unfavourable to their formation². The San Andreas fault to the north is recognised as the major part of the boundary between two large plates—the Pacific and North American. The Gulf of California is a transitional region between predominantly seafloor spreading to the south and totally transform fault motion to the north. The lack of identifiable magnetic anomalies in the gulf makes it necessary to look for other evidence to support the idea of seafloor extension. The pole of rotation for the Pacific-North American plates is 50.9°N , 66.3°W (ref. 3). The trend of the gulf differs sufficiently from a small circle about this pole to require *en echelon* offset of the transform faults and thus requires some seafloor extension in the gulf. Large heat flow⁴ and microseismicity⁵ in the basins also suggest that the gulf is undergoing extension as well as transform fault motion.

Shepard⁶ recognised the similarity between the Gulf of California and the Red Sea and mapped the *en echelon* strike-slip faults and rhombic nature of the basins. Rusnak, Fisher and Shepard⁷ related the opening in the gulf to the strike-slip motion of the San Andreas fault and deduced a figure of 260 km of opening, with Cabo San Lucas originally near Banderas Bay, Sinaloa. They considered the central depressions in the Guaymas and Farallon Basins to be extensional features due to their perpendicularity to the strike-slip faults. Larson¹ deduced a half-spreading rate of 30 mm yr^{-1} by correlating magnetic anomaly patterns at the mouth of the gulf. Lawver *et al.*⁴ reported on 13 new heat-flow measurements in the Guaymas Basin bringing the then total to 16, with three from Von Herzen⁸. Large values were found in the north-east central Guaymas Deep, $>5 \text{ HFU}$ ($>200 \text{ mW m}^{-2}$) with the greatest being 7.2 HFU (300 mW m^{-2}) near a 160-m high mound in the deep.

Measurements and techniques

The most recent cruise, in October 1974, collected 58 new heat-flow measurements in the Guaymas Basin. Most of the work

was concentrated in the north-east Guaymas Deep but some of the most interesting results came from the south-west Guaymas Deep (Fig. 1).

The thermal gradients were measured using the Woods Hole Oceanographic Institution multipenetration heat-flow probe⁹. It consists of a 2.5-m probe with three outrigger thermistor probes at 1-m intervals. Acoustically telemetered data were recorded on a precision depth recorder aboard the RV Agassiz of the Scripps Institution of Oceanography. Navigation consisted of radar fixes and depths later plotted on the satellite navigated bathymetric chart of G. F. Sharman (unpublished). The radar navigation was accurate to about 500 m, with relative positions between individual measurements being better ($\sim 250 \text{ m}$), see Fig. 2. The sediments in the Guaymas Basin are extremely uniform and consist almost solely of green hemipelagic mud. Thermal conductivities were assumed from measurements made on previous cruises⁴. The water content is very high in the near-surface sediments causing the conductivity in those shallower than 50 cm to be about $1.5 \times 10^{-3} \text{ calorie } ^\circ\text{C}^{-1} \text{ s}^{-1} \text{ cm}^{-1}$ ($0.60 \text{ W m}^{-1} \text{ K}^{-1}$). The conductivity below 50 cm is assumed to be reasonably constant, based on the findings of Lawver *et al.*⁴ and is taken to be $1.71 \times 10^{-3} \text{ calorie } ^\circ\text{C}^{-1} \text{ s}^{-1} \text{ cm}^{-1}$ ($0.71 \text{ W m}^{-1} \text{ K}^{-1}$). The heat-flow values are shown on the diagram in Fig. 3 and listed in Table 1.

The average sedimentation rate in the central part of the Guaymas Basin is approximately 2.0 m per 1,000 yr (ref. 10). This high sedimentation rate depresses the surface heat flow, requiring approximately a +12% correction according to Fig. 9 of ref. 11. From the seismic reflection profiles in Fig. 5, the south-west Guaymas Deep seems to be underlain by basement which has intruded through older sediments, as evidenced by the apparent $0.8 \pm 0.2 \text{ s}$ thick sediment to the north and $0.6 \pm 0.1 \text{ s}$ thick sediment to the south. Later we show that the heat-flow anomaly is consistent with an intrusion of this geometry with an age of about $2 \times 10^4 \text{ yr}$. For this model, the anomaly is completely dominated by the transient cooling of the intrusion and the sedimentation rate has a negligible influence. For purposes of discussion no corrections will be made to the recorded heat-flow values.

Interpretation

Nearly every other region of active seafloor generation is almost devoid of sediments making heat-flow measurements there possible only in isolated sediment ponds. The available heat-flow values from these regions are apparently depressed by hydrothermal circulation. The heat-flow values measured in the Gulf of California are significantly higher than those measured on other spreading centres. Henyey (unpublished) measured a

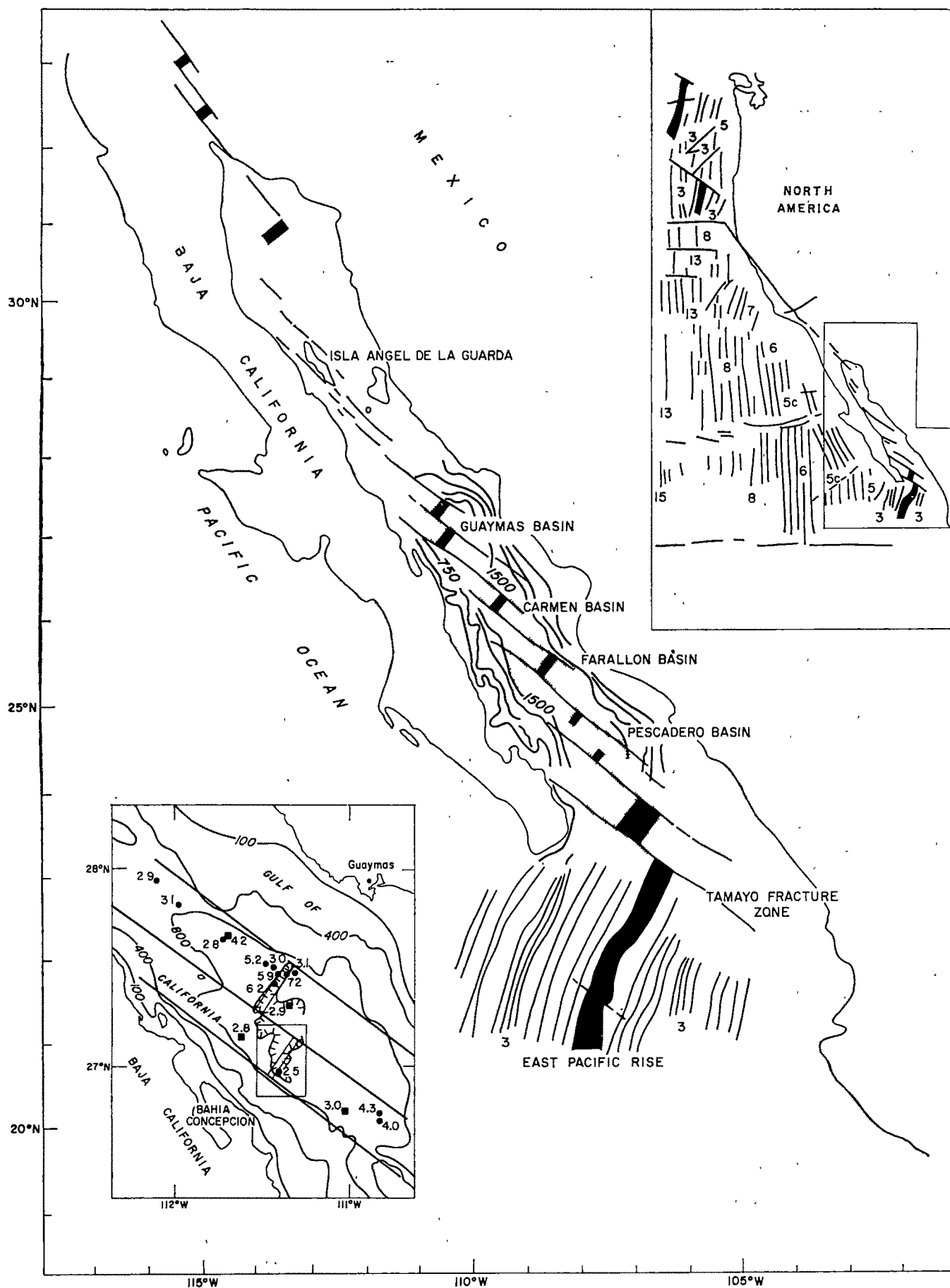


Fig. 1 Lineated magnetic anomalies at the mouth of the Gulf of California, from Larson¹. Generalised bathymetric lines in gulf in metres. Upper inset map with magnetic anomaly numbers from Atwater²⁰. The lower inset is a generalised diagram of the Guaymas Basin from Lawver *et al.*⁴ showing heat-flow values, in $\mu\text{calorie cm}^{-2} \text{ s}^{-1}$. Squares indicate values from Von Herzen⁸. Circles from Lawver *et al.*⁴. Box shows area of detailed heat-flow measurements shown in Fig. 2. Heavy lines and stippled areas mark transform faults and areas of active generalised extension, respectively.

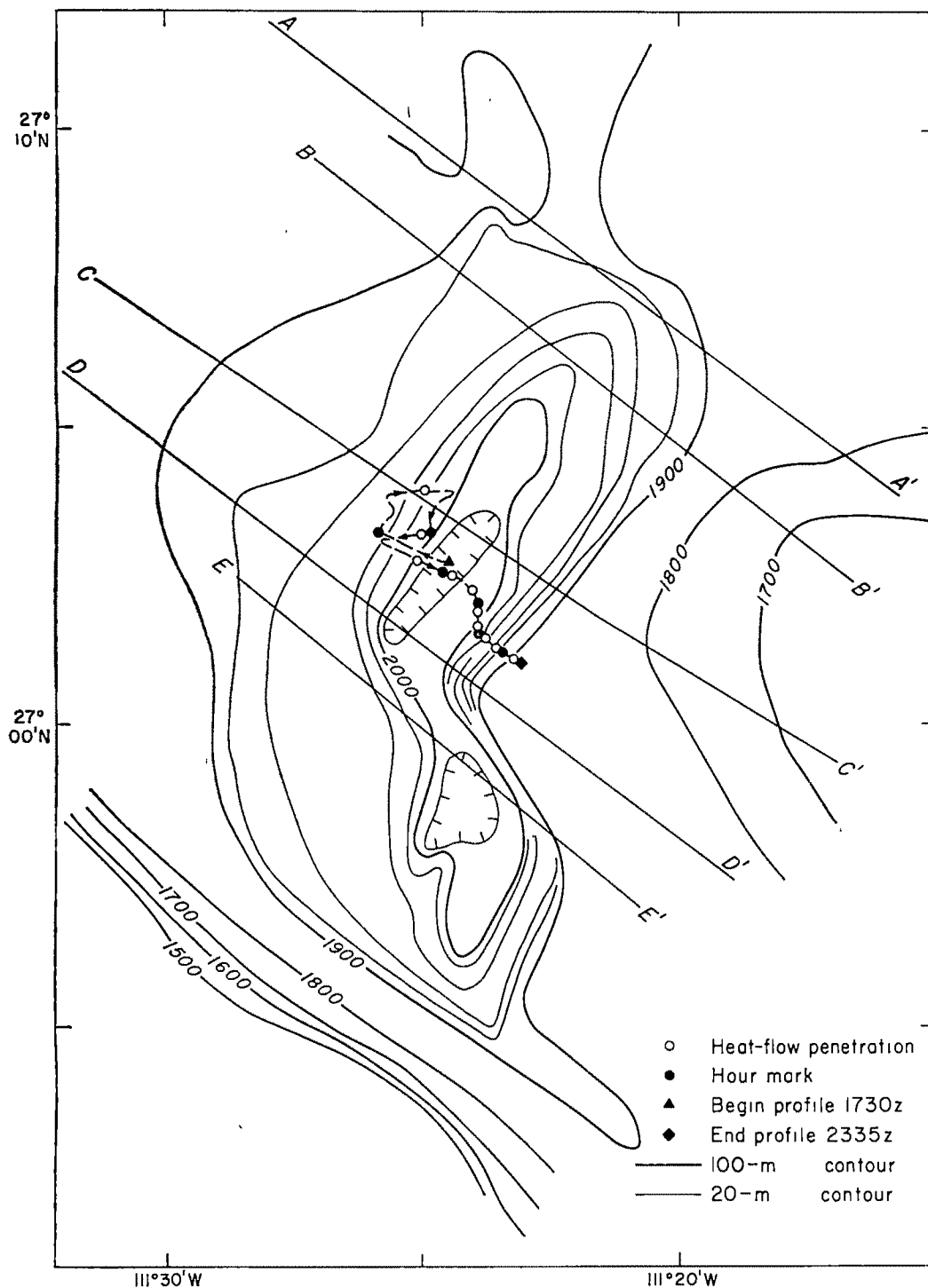


Fig. 2 Detailed chart of heat-flow survey of the south-west Guaymas Deep in corrected metres. Bathymetry from G. Sharman (unpublished). The values progressed from the north-west side of the depression to the south-east side. Seismic reflection profiles A-A' to E-E' indicated by solid lines are shown in Fig. 5.

value of 35.8 HFU ($1,450 \text{ mW m}^{-2}$) in the Ballenas Channel in the northern gulf but this may be an isolated hot spring.

The heat release associated with lithospheric creation for a spreading rate equivalent to that of the gulf is estimated to be at least 330 calorie s^{-1} per cm of ridge length within 35 km of the spreading centre¹² or an average heat flow of 47 HFU. At most oceanic spreading centres the rate is presumed much higher near the centre due to the faster cooling produced by hydrothermal circulation (compare ref. 12). The Gulf of California is different because the actively spreading zone may be losing heat through the sea floor to a greater extent by conduction because of its unusually thick and continuous sedimentary cover.

Figure 4 shows all the 26 published and new heat-flow values in the Guaymas Basin plotted against distance from the presumed most recent spreading centre. Additional heat-flow

values in the Guaymas Basin to be discussed in a subsequent paper reinforce the basic shape of the curve in Fig. 4. It is unusual that the background heat flow for the Guaymas Basin is remarkably regular at 3.4 ± 0.5 HFU for the total length of the basin. The central deep coincides with a higher heat-flow zone less than 20 km wide.

This distribution of conductive heat flow does not fit the theoretical models for a conductively cooled lithosphere (see ref. 13). As can be seen in Fig. 4, the observed heat-flow values average less than half of the predicted value. Two factors, prevalent in the Gulf of California, would tend to make the measured values lie below the theoretical curve. First, the theoretical models assume continuous intrusion whereas in the Gulf of California there is evidence that the location of spreading centres changes discontinuously with time. This idea was first advanced by Bishoff and Henyey¹⁴ on

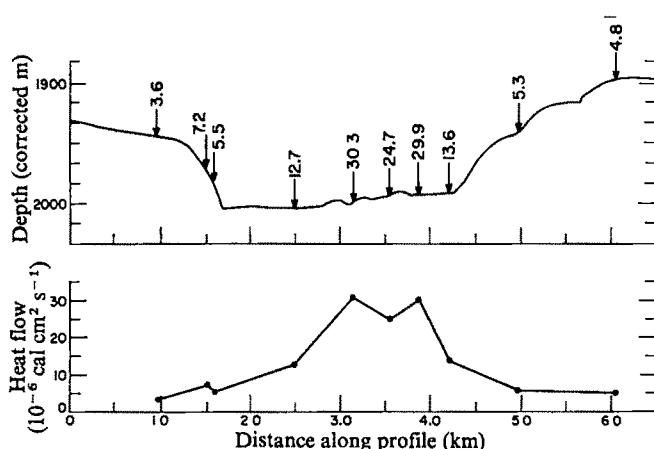


Fig. 3 Profile across the Guaymas Deep showing the heat-flow (HFU) values and the shape of the anomaly found.

the basis of seismic reflection profiles. It is supported by our heat-flow measurements and the reflection profiles shown in Fig. 5. At (a) on profile D-D' (Fig. 5), there is a slight depression which has not been completely filled with sediment; it has an uncertain age relationship to the central intrusion. Probably the depressions are in fact grabens caused by the pulling apart of the basement, resulting in an intrusion and a thinning of the overlying sediment (Fig. 3, ref. 21). The weight and shear strength of the sediments compared with water may cause the intrusion to slow and stop before it reaches the surface. This effect of the sediment may even cause the site of intrusion to change discontinuously rather than remaining fixed as at a normal spreading centre. The second factor that may affect the heat flow is that the ratio of the widths of spreading centres to the length of transform faults is much less in the Gulf of California than in more normal oceanic spreading areas. This could lead to significant lateral heat transfer. Although these factors redistribute heat to the flanking regions and away from the actively spreading locations, the total heat loss remains approximately the same and thus will not explain the great discrepancy between the predicted and observed heat flows.

Assuming that seafloor spreading applies in the gulf, the most plausible explanation for the discrepancy is hydrothermal heat loss even though there is a thick sediment cover. The existence and importance of hydrothermal convection in the basement have been discussed by numerous investigators (see reference lists in refs 12 and 15). The porosity and permeability would be related to dike contacts, the dissolution of minerals by thermal waters, the horizontal component of thermal contraction, and faulting. In the gulf the evidence is not as compelling as it is at other spreading centres. There is no evidence of numerous low heat-flow values and the large scatter in ob-

served values that have supported previous hydrothermal circulation hypotheses. We made approximately 50 km of near-bottom horizontal water temperature profiles (see ref. 12) in the north-east Guaymas Basin but failed to detect any temperature anomalies that might be associated with hydrothermal vents. Evidence for sediment that was more metalliferous than normal was found only in the southernmost basin of the gulf (P. Wilde, personal communication). Since this basin is nearest to the East Pacific Rise and has a much thinner sediment cover¹⁸ it is more likely to be subject to hydrothermal discharge through the sediment surface. In the heavily sedimented Guaymas Basin, where the sediments are assumed to be relatively impermeable and there is very little expression of faulting in the surface sediments, it is hard to imagine the free convection that Williams *et al.*¹² found on the Galapagos spreading centre.

Thermal modelling

With the line drawings of seismic reflection profiles crossing the south-west Guaymas Deep (shown in Fig. 5), available as control, the heat-flow data were inverted under the assumption of conductive cooling. We first used Horai's model¹⁷ for a dike intruded instantaneously and remaining at a constant temperature for infinite time. This model yields a 1.6-km wide intrusion with a depth to its top of 220 ± 30 m, using a background heat flow of 3.4 ± 0.5 HFU. But this gives either an unacceptably high surface thermal gradient (5°C cm^{-1}) or a temperature too low for molten rock (400°C). This result implies that the measured heat-flow values are not due to a constant temperature intrusion but rather to a transient cooling process. Therefore, we used Simmons¹⁸ method for modelling the transient cooling of a dike, which is a variation of that given by Carslaw and Jaeger (ref. 19, p. 256). The temperature for a dike is given as:

$$T(x, y, z, t) = (S/8) E(x, x_1, x_2) E(y, y_1, y_2) \{ E(z, z_1, z_2) + E(z, -z_1, -z_2) \}$$

where S = source strength, which for a three-dimensional source is simply the temperature at the time of the intrusion, here assumed to be $1,100^\circ \text{C}$; x_1 and x_2 are coordinates defining the width of the dike; y_1 and y_2 give the lateral extent of the dike; z_1 and z_2 are the depths to the top and bottom respectively and E is a dimensionless function defined as:

$$E(\xi, \xi_1, \xi_2) = \text{erf} \frac{\xi - \xi_1}{(4kt)^{1/2}} - \text{erf} \frac{\xi - \xi_2}{(4kt)^{1/2}}$$

where t is the time after intrusion and k is thermal diffusivity. If one assumes y_1 and y_2 are large, greater than $3(4kt)^{1/2}$, then $E(y, y_1, y_2)$ is two. For a time of 10^4 yr the absolute value of y_1 or y_2 would have to be larger than 0.8 km. Using Figs 2 and 5, it seems that $y_1 = -2.0$ km and $y_2 = 6.0$ km. z_2 is the

Table 1 Heat-flow data

Station no.	Latitude (°N)	Longitude (°W)	Water depth (corrected m)	T	P	N	K	Q
12.1	27°04.0'	111°24.4'	2,004	2.82	2.7	3	1.71 (0.72)	5.5 (230)
12.2	27°03.6'	111°24.9'	1,988	2.82	2.7	3	1.71 (0.72)	7.2 (301)
12.3	27°03.1'	111°25.8'	1,955	2.82	2.7	3	1.71 (0.72)	3.6 (151)
12.4	27°02.6'	111°24.6'	2,024	2.83	2.7	2	1.71 (0.72)	12.7 (531)
12.5	27°02.4'	111°24.3'	2,020	2.82	2.7	1	1.71 (0.72)	30.3 (1268)*
12.6	27°02.2'	111°24.0'	2,014	2.82	2.7	1	1.71 (0.72)	24.7 (1033)*
12.7	27°02.0'	111°24.0'	2,012	2.82	2.7	1	1.71 (0.72)	29.9 (1251)*
12.8	27°01.8'	111°23.9'	2,010	2.82	2.7	3	1.71 (0.72)	13.6 (569)
12.9	27°01.5'	111°23.7'	1,952	2.83	2.7	3	1.71 (0.72)	5.3 (222)
12.10	27°01.3'	111°23.4'	1,898	2.83	2.7	3	1.71 (0.72)	4.8 (201)

T is bottom water temperature ($^\circ \text{C}$); P is estimated sediment penetration (m) of the lowermost probe used in gradient measurements; N is the number of thermistors used for sediment temperature gradient measurements; K is the thermal conductivity in 10^{-3} calorie $^\circ \text{C}^{-1} \text{cm}^{-1} \text{s}^{-1}$ ($\text{W m}^{-1} \text{K}^{-1}$) (All values are assumed); Q is the heat flow in 10^{-6} calorie $\text{cm}^{-2} \text{s}^{-1}$ (10^{-3} W m^{-2}).

*Only one thermistor on scale.

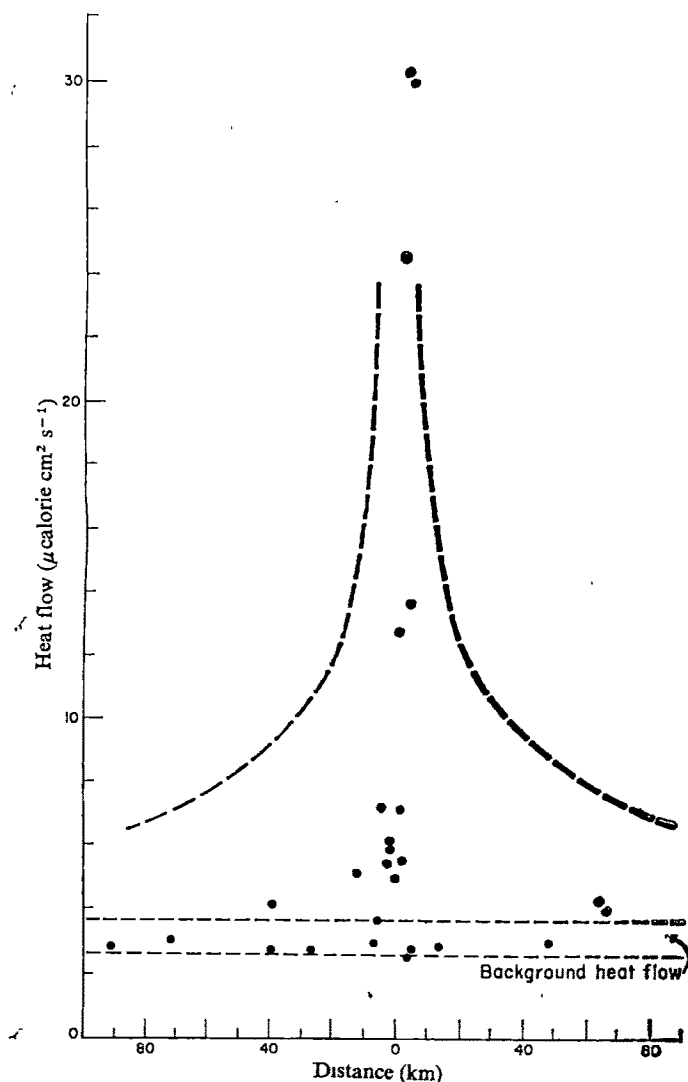


Fig. 4 Summary of the previous published values and the ten new values. The background heat flow is roughly 3.4 ± 0.5 HFU (135 ± 20 mW m $^{-2}$). The heat flow anomaly is strikingly regular directly over the depressions confirming them as the locus of present intrusive activity.

depth to the base of the dike which we assume is large in comparison to z_1 . If z_2 is just twice z_1 , it affects Q_z only 2%.

Simmons (ref. 18, equation 17) gives the surface heat-flow distribution as:

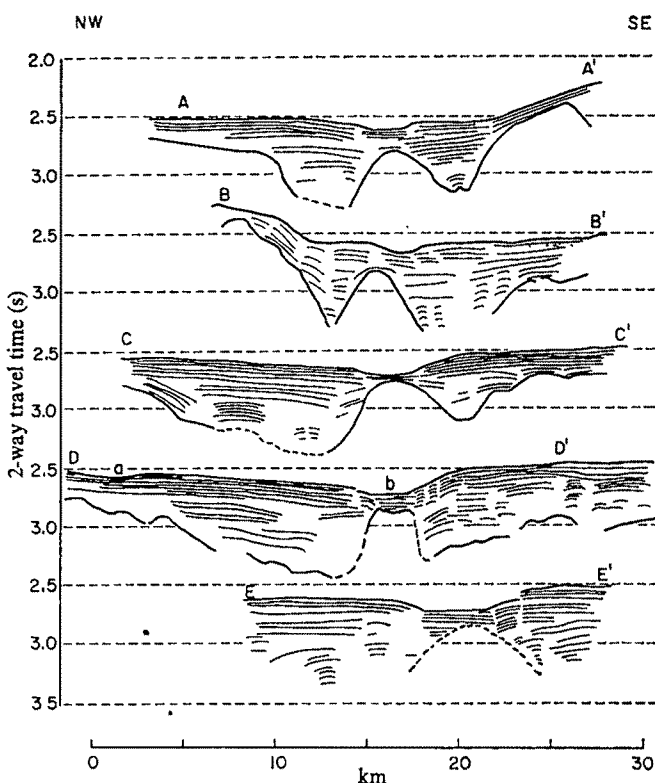
$$Q_z \big|_{z=0} = \frac{-KS}{2(\pi kt)^{1/2}} E(x, x_1, x_2) [\exp(-z_1^2/4kt) - \exp(-z_2^2/4kt)]$$

In this model, the same thermal properties are assumed for the dike as for the material into which it is intruded. We assume the thermal properties of sediments to be more realistic because the cooling is probably dominated by the insulating properties of the sediment. But the igneous body should be able to release heat faster, and should be younger than our solutions indicate. In Fig. 6 however, we show that both sediment and igneous rock thermal properties can be made to match the data. We have taken a conductivity, K , of 2×10^{-3} calorie °C $^{-1}$ s $^{-1}$ cm $^{-1}$ (0.83 Wm $^{-1}$ K $^{-1}$) and diffusivity, k , of 2.35×10^{-3} cm 2 s $^{-1}$ to approximate a slightly compacted sediment column²⁰. Even greater values might be expected considering the probable metamorphism of the sediments directly above the intruded body. From the seismic reflection profile D-D' shown in Fig. 5, it seems that the anomaly is due to an intrusion

that is directly underneath the depression. From Fig. 5 the intrusion seems to be present and approximately the same shape on profiles A-A' through D-D' and is vague but present on E-E'. The intrusion seems to extend from one transform fault to the other. On profile D-D', closest to the profile of heat-flow stations, the depth to the top of the intrusion seems to be about 120–200 m. Figure 6 shows that using a depth of 200 m and removing the background heat flow, our anomaly is closely matched with a width of 1.3 km, and an age of 17,000 yr. For this model a maximum heat flow of 53 HFU would be reached at about 2,700 yr after the emplacement of the intrusion. The observed heat-flow maximum of 30 HFU is found twice for any model that assumes $z=200$ m, once at about 1,000 yr and again at 17,000 yr. The earlier time value seems unacceptable because it produces a very steep-sided anomaly as shown in Fig. 6, unlike the profile measured. If one assumes a depth to intrusion of 100 m, then an age of 18,000 yr very nearly models the observed anomaly. A greater depth to the intrusion is more difficult to model with thermal properties of sediments, although the thermal properties of the intrusive rock will fit the observed curve for a depth to intrusion of slightly greater than 500 m.

The seismic reflection profile indicates that the base of the intrusion may be 4 km in width, equivalent to about 65,000 yr of spreading at the half-rate of 30 mm yr $^{-1}$. It seems plausible to assume that the most recent intrusion is simply the latest in a continuing series of recent intrusions now totalling 4 km. If the most recent intrusion was emplaced into an older intrusion not at thermal equilibrium then our model would be less representative and much more recent ages of intrusion are possible. A 1.3 km wide intrusion would account for 21,000 yr of spreading. Since we have more than 42 m of sediment (2 m per 1,000 yr for 21,000 yr), we assume that the intrusion has always been covered. Hydrothermal cooling which might account for removal of about half the heat would be roughly equivalent to doubling the thermal conductivity (similar to the conductivity

Fig. 5 Line drawings of seismic reflection profiles. Line of profiles shown on Fig. 2. In profile D-D', *a* indicates a possible depression that is either just forming or is being filled and *b* indicates the area of the main heat-flow anomaly.



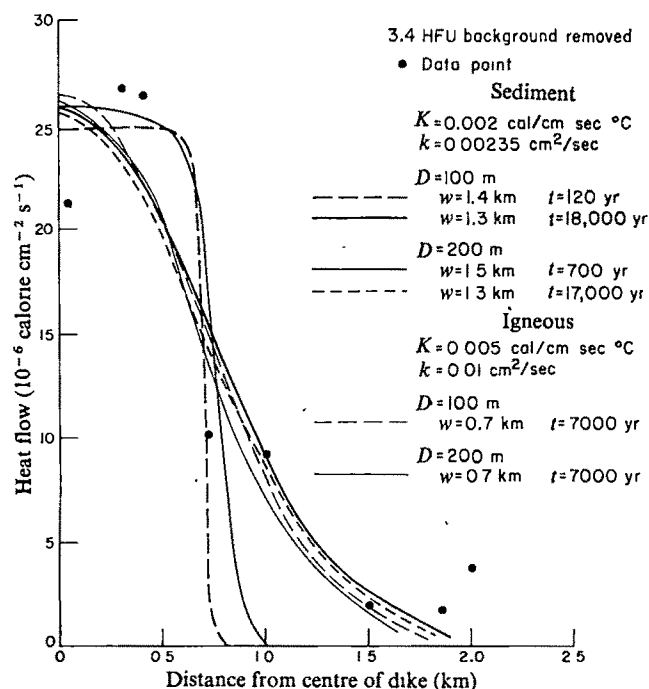


Fig. 6 Results of Simmon's¹⁸ model for heat-flow produced by a cooling intrusion.

of the intrusive rock). Although there are many variable parameters not defined by our data, the results from the model can be summarised as indicating an intrusion approximately 1.0 km wide, beneath 150 ± 100 m of sediments and less than 18,000 yr old.

Conclusions

Our findings allowed us to use heat-flow measurements to model a reasonably young intrusion, something that is rarely achieved. It does seem reasonably certain from our results that the gulf is not being opened by a continuing constant intrusion as is presumed at most oceanic spreading centres. Discontinuous and episodic intrusions into a thick sedimentary cover would explain the lack of magnetic anomalies in the gulf. Apparently the intrusion is continuous between transform faults and is believed contemporaneous.

The Gulf of California has an average sediment depth of

about 1 km (ref. 16). This, coupled with the high background heat flow, suggests that temperatures of 200 °C should generally be found near the sediment-rock interface. This is in addition to large areas where the thermal gradient is several times as high. If the upper regions of the basement are as porous and permeable as we believe, the Gulf of California would be one of the Earth's most important geothermal resources. This theory, though, can only be tested by drilling.

More measurements are needed in this area to trace the assumed lateral extent of the anomaly. Since the intrusion seems to be continuous between the transform faults, one would hope that the heat flow remains uniformly high in this region. Other sites in the Guaymas Basin and particularly the Farallon Basin to the south may be undergoing current intrusion and should be investigated.

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Three-dimensional model of purple membrane obtained by electron microscopy

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A 7-Å resolution map of the purple membrane has been obtained by electron microscopy of tilted, unstained specimens. The protein in the membrane contains seven, closely packed, α -helical segments which extend roughly perpendicular to the plane of the membrane for most of its width. Lipid bilayer regions fill the spaces between the protein molecules.

THE purple membrane is a specialised part of the cell membrane of *Halobacterium halobium*¹. Oesterhelt and Stoekenius² have

shown that it functions *in vivo* as a light-driven hydrogen ion pump involved in photosynthesis. It contains identical protein molecules of molecular weight 26,000, which make up 75% of the total mass, and lipid which makes up the remaining 25% (ref. 3). Retinal, covalently linked to each protein molecule in a 1:1 ratio is responsible for the characteristic purple colour³. These components together form an extremely regular two-dimensional array⁴.

We have studied the purple membrane by electron microscopy using a method for determining the projected structures of unstained crystalline specimens⁵. By applying the method to tilted specimens, and using the principles put forward by

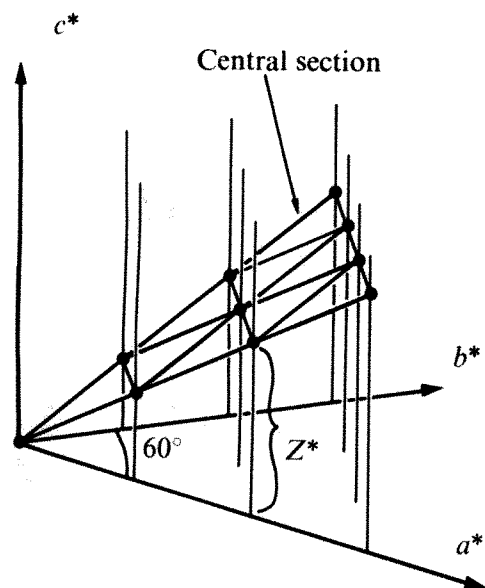


Fig. 1 Part of the three-dimensional reciprocal lattice showing the geometry of the lattice lines in the hexagonal space group P3. a^* , b^* and c^* are the reciprocal lattice vectors. a^* and b^* lie in, and c^* is perpendicular to the plane of the membrane. A central section which is perpendicular to the incident electron beam has been drawn through the lattice. The intersection of this central section with the reciprocal lattice is determined by the angle of tilt and the axis about which the membrane is tilted. Individual diffraction patterns and micrographs provide the amplitudes and phases in this section at the points shown. z^* represents the coordinate along the c^* direction of one of the points. The angle of tilt was measured to within 2° for each of the specially modified, tilted specimen holders, and the direction of the tilt axis on the photographic plate was established during operation of the microscope. However, estimates based on the geometry of the spacings of the lattice points (for high tilt angles), the variation of the degree of underfocus across the plate (for low tilt angles), and least squares refinement against data obtained at high tilt angles (for diffraction patterns) provided more accurate figures which were used in the calculation. The accuracy of measurement of both the amplitudes and phases depended on having sharp lattice lines. We therefore took care to ensure that, on the microscope grid, the membranes remained coherently ordered and flat to within $1/5^\circ$.

De Rosier and Klug⁶ for the combination of such two-dimensional views, we have obtained a three-dimensional map of the membrane at 7 Å resolution. The map reveals the location of the protein and lipid components, the arrangement of the polypeptide chains within each protein molecule, and the relationship of the protein molecules in the lattice.

Electron microscopy and diffraction

The purple membrane was prepared under normal conditions from cultures of *H. halobium*³ and applied to the microscope grid in the presence of 0.5% glucose. The purified membranes are mostly oval sheets up to 1.0 μm in diameter and about 45 Å thick^{4,7}. The array of molecules making up these sheets is accurately described⁷ as an almost perfect crystal of space group P3 ($a = 62$ Å) with a thickness of one unit cell only in the direction of the c axis. A single membrane thus contains up to 40,000 unit cells; that is 120,000 protein molecules (three per unit cell).

These large periodic arrays from which electron diffraction patterns and defocused bright field micrographs are recorded⁵ enable us to overcome the principal problem normally associated with high resolution electron microscopy of unstained biological materials; that is, sensitivity to electron damage⁸. Only a small number of electrons can pass through each unit cell before it is destroyed, but because of the large number of unit cells, the information in the diffraction patterns and micrographs is sufficient to provide a picture of the average

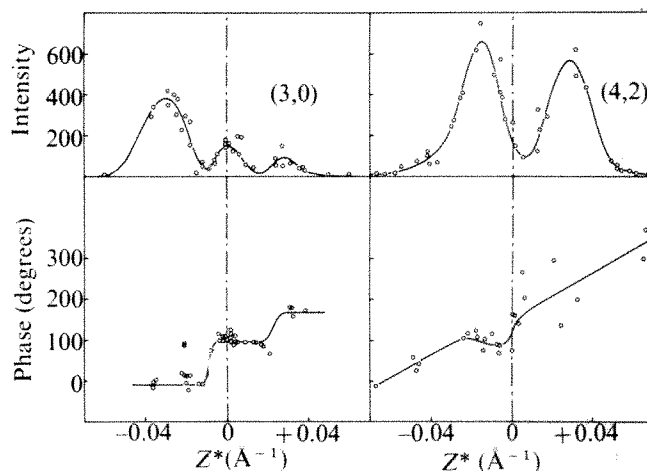
unit cell. The micrographs recorded with such low doses of electrons appear featureless, since the statistical fluctuation in the number of electrons striking the plate is large compared with the weak phase contrast ($<1\%$) produced by defocusing. As a result, analysis of each micrograph by densitometry and computer processing⁵ is required to combine the information from individual unit cells.

Solution of the three-dimensional structure of the purple membrane requires the determination of the amplitudes and phases in three dimensions of the Fourier terms into which it can be analysed. The diffraction pattern or Fourier transform of the membrane is not a three-dimensional lattice of points as is the case with a normal crystal, but since it is only one unit cell thick, a two-dimensional lattice of lines which are continuous in the direction of c^* (that is perpendicular to the membrane). A single electron diffraction experiment therefore gives rise to a two-dimensional pattern of spots which correspond in their intensities to the square of the amplitudes of the transform along a central section through the three dimensional transform of the membrane (Fig. 1). Similarly, an electron micrograph contains information about the phases (and about the amplitudes, but less accurately than does the diffraction pattern) in the central section. Previously⁶, we were able to determine the projected structures of both the purple membrane and thin catalase crystals from the amplitudes (obtained by electron diffraction) and phases (by Fourier analysis of micrographs) along the single central section through the transform parallel to the plane of the object.

Here, we have collected data similar to those obtained previously but from 15 diffraction patterns and 18 micrographs (actually pairs of micrographs, see ref. 5) of membranes tilted at angles from 0° to 57° to the incident electron beam, to determine the amplitudes and phases at a number of points along each of the lattice lines. These data provided estimates of the continuous variation of the transform along the lines. By sampling the continuous curves at appropriate intervals, it was then possible to calculate the structure as if for a three-dimensional crystal with some arbitrary c -axis dimension greater than the membrane thickness. This method of combining two-dimensional images was proposed originally by De Rosier and Klug⁶ for a general object, although in this case we have made use of electron diffraction patterns as well.

The diffraction patterns provided 1,800 independent intensity measurements which were combined to give continuous curves, such as those shown in Fig. 2, for each of the 36 crystallographically independent lattice lines out to a resolution of 7 Å.

Fig. 2 Values of intensity and phase along two lattice lines, obtained respectively from the diffraction patterns and images using the geometry shown in Fig. 1. Smooth curves were drawn through the points to give reliable intensities and phases at suitably fine sampling intervals (0.01 Å^{-1}) in z^* . These values were used to compute the three-dimensional structure.



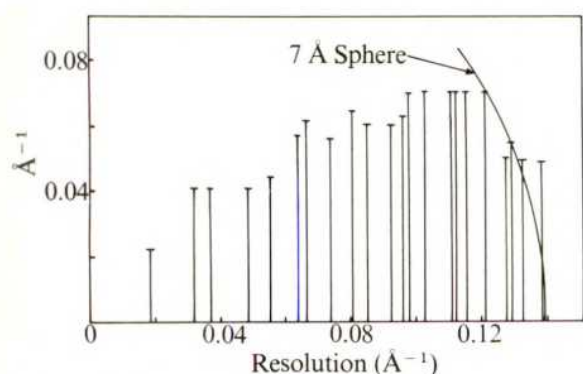


Fig. 3 Schematic diagram showing the region of reciprocal space contributing to the three-dimensional Fourier map. Part of the boundary of the 7 Å sphere is indicated. The region shown, which includes 63% of the total volume of this sphere, includes almost all the strong diffraction peaks to 7 Å, with the principal exception of the axial (0,0) lattice line (but see text).

Because of the distribution of intensity in the transform⁷, due to real features in the structure, very little diffracted intensity (none greater than 1/50th of the strongest intensity) occurred at values of z^* greater than 0.08 Å^{-1} for any lattice line.

With the electron micrographs compensation for the effects of defocus was rather more complicated than in the case of untilted specimens⁸. Since the membranes we studied were at least 5,000 Å in diameter, different parts of the membrane in a tilted specimen were situated at significantly different distances from the objective lens. As a result, the phase contrast transfer function, which depends on the degree of underfocus of this lens, varied across the image. The correction of the contrast of the image could not be made after transforming the image by altering the phases of the Fourier components of the image, as was done previously, but instead had to be carried out at an earlier stage, so that different parts of the image where the contrast is positive or negative could be made to combine constructively, and thereby yield, on transformation, the correct phases for the object. This procedure was conveniently carried out between the execution of the first and second dimensions of the Fourier transformation having ensured that the images were densitometered parallel to the tilt axis. The contrast along each scan line could then be corrected by multiplying the one-dimensionally transformed data by the contrast transfer function, $-2 \sin \chi$ (see ref. 5) for each reflection, so that the computed phases had the correct sign for the object, and gave the maximum signal-to-noise ratio. The phases were then combined image by image after refining the phase origin of each image so that the best agreement of its phases with all previously accumulated phases was obtained. By starting with the untilted data with the phase origin at one of the threefold positions, and adding the phases from images with smaller tilt angles first, the maximum accuracy in combining the phases was ensured. This procedure also meant that the phase origin for the combined three-dimensional data ended roughly in the middle of the membrane on one of the threefold axes. The average phase error in a single measurement estimated from the differences between those of the 1,000 independently recorded phases having similar values of z^* was less than 20° , as found previously⁵. As with the intensity data, continuous curves could be drawn through these phase measurements (Fig. 2).

The intensity and phase curves were sampled at intervals of 0.01 Å^{-1} to provide Fourier terms from which to calculate the three-dimensional map. This sampling interval is more than sufficiently fine to ensure an accurate representation of a structure only 45 Å thick.

Three-dimensional potential map

The map (Fig. 4) was a Fourier synthesis of 365 crystallographically independent terms which extended to a resolution

of 7 Å in the plane of the membrane, but which included only terms with spacings greater than 14 Å perpendicular to it. There were two reasons for the asymmetrical distribution of Fourier terms. First, the distribution of intensity in the transform of the membrane is such that the diffraction beyond spacings of 7 Å in the plane of the membrane, and about 20 Å perpendicular to it, is weak and therefore difficult to measure with the limited total diffraction available from membranes only $1 \mu\text{m}$ in diameter. The average intensity in these regions by X-ray diffraction⁷ is 7–10 times lower than the in-plane diffraction near 10 Å , and by electron diffraction we find no intensity greater than 1/50th of the strongest diffraction at values of z^* greater than 0.080 Å^{-1} . Second, the use of tilt angles up to some limiting angle, in this case 57° , meant that a conical region of reciprocal space was unmeasured. The volume of reciprocal space finally included is shown schematically in Fig. 3. Although a considerable volume of the 7 Å sphere is missing (37%) the effect of its inclusion in the map would be very small since the X-ray diffraction pattern indicates that the amplitudes of the Fourier components in the missing regions are small. Therefore, since the map includes all the strong terms present to 7 Å resolution, it provides an accurate representation of the dominating features of the structure to this resolution.

As a further check on the effect of leaving out some of the near axial reflections, a second map was calculated which included the profile diffraction determined by X rays. The amplitudes were obtained from the published X-ray profile

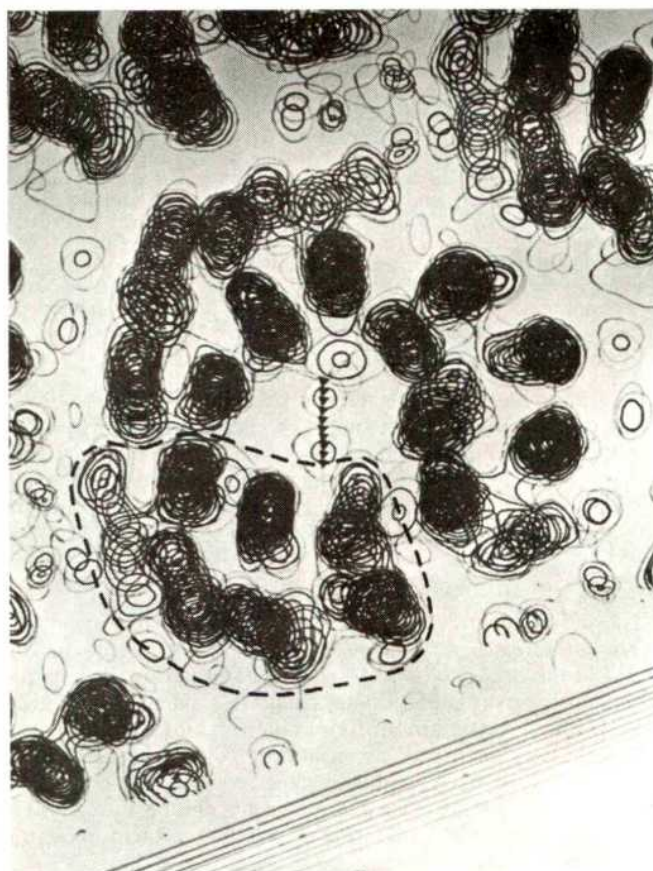


Fig. 4 Part of the three-dimensional potential map showing a region 50 Å thick spanning the membrane. The sections above and below this region contain no features higher than one contour level. The absolute hand is determined by the known direction of specimen tilt. The view shows protein molecules grouped around one of the threefold axes. The probable boundary of one of them is indicated by the broken line. The angle of view is such that the α helices furthest from the threefold axis partly overlap in the protein molecule outlined, but are seen almost end-on in the one on the right.

of Blaurock and Stoeckenius⁴ by sampling at 0.01 \AA^{-1} intervals, and scaled to our data by comparing the profile intensity with that of the (1,1) ring in an unoriented X-ray powder pattern. The phases were assumed to be those of a symmetrical membrane⁴ with the origin in the middle. The inclusion of the Fourier terms out to 0.05 \AA^{-1} made little difference to the map but, as would be expected, slightly raised the overall level of the contours on each side of the membrane (by about one contour level). The axial (0,0) line is by far the strongest region not included in our map, so clearly the effect of excluding the remaining unmeasured volume of reciprocal space is small.

Since the amplitudes and phases used to calculate the present map are derived from the scattering of electrons, the physical quantity which it represents is, strictly, the electrical potential inside the membrane. At this resolution, however, the potential is roughly proportional both to the electron density, as would be found for instance by X-ray analysis, and to the atomic number. High density features therefore correspond to the presence of denser groupings of atoms.

Structure of the membrane

The appearance of the map is dominated by numerous rod-shaped features aligned perpendicular to the plane of the membrane. There are seven rods in each asymmetric unit of the crystal and they are packed 10–12 \AA apart. Adjacent rods are slightly inclined to one another at various angles from 0° to 20° and they are all roughly 35–40 \AA long. In view of the existence^{7,9} in the X-ray diffraction pattern of strong axial reflections at 1.5 \AA and 5 \AA which are characteristic of the α -helix, there is little doubt that the seven rod-shaped features in the map are α -helices which extend perpendicular to the membrane for most of its width. Since the protein has a molecular weight of 26,000 (ref. 3), it follows that the α -helices make up 70–80% of the polypeptide. The connectivity of the helices, however, cannot be discerned unambiguously at this resolution.

The tentative boundary of an individual protein molecule indicated in Fig. 4 is the one most likely to be correct since it surrounds regions of the protein with maximum connectivity and it is this part of the map which we have used to make the model shown in Fig. 5. The overall dimensions of the protein in our model are $25 \times 35 \times 45 \text{ \AA}$, with the longest dimension perpendicular to the plane of the membrane and parallel to the helices. The three protein molecules which are most intimately in contact are grouped round the threefold axis in an interesting manner. Three of the seven α helices in each protein molecule are nearer the centre of the group giving an inner ring of nine helices which are all 10 \AA (± 1) apart and in contact with one another. The other four helices in each protein combine to make an outer ring of twelve which surround the inner nine. The outer helices are all slightly more inclined to the perpendicular than the inner ones, and are not all in contact. The three most strongly tilted of these helices were not resolved in projection⁵, since they partly superimpose when viewed perpendicular to the plane of the membrane. The direction of tilting of the outer helices is consistent with the interlocking of the amino acid side chains from adjacent helices—that is the structure is a left-handed supercoil, as expected for a right-handed α helix¹⁰.

In the space in the middle of the ring of nine helices, we presume that a small number of lipid molecules is arranged in the classical bilayer configuration. This space has a diameter of about 20 \AA ; it is not a hole filled with solvent since a difference map using X-ray intensities from wet versus dry membranes is featureless. Evidence obtained from another difference map shows that UO_2^{+2} ions can bind to this region of the membrane, again suggesting that the space is filled with lipid. Similar arguments that a lipid bilayer must be present apply to the rather even density regions which make up the remainder of the membrane structure, filling up the space between the clusters of three protein molecules.

At this resolution we cannot detect the covalently linked



Fig. 5 A model of a single protein molecule in the purple membrane, viewed roughly parallel to the plane of the membrane. The top and bottom of the model correspond to the parts of the protein in contact with the solvent, the rest being in contact with lipid. The most strongly tilted α helices are in the foreground.

retinal molecule, which is thought, from measurements of the dichroism at 560 nm in oriented specimens⁴, to be aligned parallel to the plane of the membrane—that is, perpendicular to the helices.

The structure of the purple membrane protein and the way it combines with lipid to form a two-dimensional mosaic in which the protein and lipid pack neatly side by side, each with a thickness of about 45 \AA , gives experimental support to recent concepts^{11,12} of membrane structure based on less direct evidence. In particular, the protein is globular, is almost certainly exposed on both sides of the membrane, and is surrounded by lipids which are arranged in separate areas with a bilayer configuration. The purple membrane thus seems to provide a simple example of an 'intrinsic' membrane protein, a class of structure to which many molecular pumps and channels must belong. We would not be surprised if the simple arrangement of helices found here also occurs in some of these other intrinsic membrane proteins.

A full account of this work will be published elsewhere. We thank Dr J. Pilkington and the Royal Greenwich Observatory for help with and facilities for the densitometry of the micrographs, Chris Raeburn for modifying the specimen holders and Drs L. Amos, T. Horsnell, R. Ladner and T. Takano for computer programs. We also thank our colleagues in this laboratory, particularly Dr A. Klug, for comments on the manuscript.

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letters to nature

Observations of X rays from near NGC6440

ON nine separate occasions between October 27, 1971, and January 6, 1973, the MIT instrument aboard OSO-7 scanned the region of sky within about 10° of the galactic centre. Subsequent analysis of this data, in the form of all-sky maps, led to the discovery of several previously unreported X-ray sources¹. The most intense of these, MX1746–20, was found at $\alpha = 17^h 46.0^m$, $\delta = -20^\circ 22.2'$ (1950.0) or, in galactic coordinates $l'' = 7.73^\circ$, $b'' = 3.76^\circ$. The 90% confidence contour for this position is a circle of radius approximately 0.26° . This uncertainty includes an estimate of the probable aspect error as well as statistical uncertainty.

The MIT instrument² consisted of two banks of five proportional counters sensitive from 1–60 keV. The mean intensities for each of the scans of the position of MX1746–20 are given in Table 1 for each of the four proportional counters for which useful data were obtained. The data for the low-energy counters are omitted in some cases because of contamination by solar X rays or because of the failure of one of the two lowest energy counters after August 2, 1972.

MX1746–20 is a highly variable X-ray source on time scales of months. Variations of at least a factor of 20 are observed. We have seen only weak evidence for variation on shorter time

of globular clusters as a function of angular separation from the galactic centre, we determined the probability of a chance coincidence of MX1746–20 with any globular cluster to be about 1%.

The distance to NGC6440 has not been well established although it is likely to be found close to the galactic centre. This hypothesis is supported by the galactic coordinates of the source and by its late spectral type (G5) which is believed to be typical of clusters around the centre of the Galaxy³. Assuming a distance of 10 kpc to MX1746–20, we derive a peak 1–10 keV X-ray luminosity of 3.4×10^{37} erg s⁻¹.

Like the previously discovered globular cluster X-ray sources^{3,5,6}, MX1746–20 is highly variable and its counterpart, NGC6440, is concentrated (concentration class V). This latter characteristic of the cluster adds credence to those theories of globular cluster X-ray emission which require high central densities to encourage the formation of binary systems (ref. 7 and unpublished work by A. C. Fabian *et al.*), or to provide a high enough escape velocity to cause a gas accretion rate sufficient to power the observed X-ray production from the vicinity of massive black holes (ref. 8 and J. Silk and J. Arons, unpublished).

Since all of the cluster X-ray sources discovered thus far are variable³, it may be that many more remain to be found. At

Table 1 X-ray flux of MX1746–20

Date	Intensity* (counts s ⁻¹)			
	1.0–1.5 keV	1–6 keV	3–10 keV	15–40 keV
October 27–30, 1971	1.0 ± 1.2	0.5 ± 1.0	0.8 ± 0.7	-0.1 ± 1.0
December 02–06, 1971	—	—	2.5 ± 1.0	0.3 ± 0.3
January 01–06, 1972	1.9 ± 0.7	16.4 ± 0.7	9.7 ± 0.4	0.3 ± 0.4
June 01–04, 1972	1.4 ± 2.1	-0.5 ± 2.1	0.9 ± 1.5	0.1 ± 1.9
June 30–July 09, 1972	-0.2 ± 0.2	1.6 ± 1.0	1.1 ± 0.4	0.4 ± 0.3
August 28–31, 1972	—	3.2 ± 1.2	1.8 ± 0.5	0.4 ± 0.3
September 14–20, 1972	—	4.2 ± 1.5	2.9 ± 0.7	0.7 ± 0.4
December 03–09, 1972	—	—	0.9 ± 0.4	0.4 ± 0.2
January 02–06, 1973	0.2 ± 0.5	-0.7 ± 0.8	-0.4 ± 0.3	0.4 ± 0.4

*The observed intensities may be converted to photon fluxes by the approximate formula $\Phi = \gamma/I$ where Φ = flux in photons cm⁻² s⁻¹ keV⁻¹, I = intensity in counts s⁻¹, and $\gamma = 0.15$ (1.0–1.5 keV), 0.0069 (1–6 keV), 0.0045 (3–10 keV), 0.0013 (15–40 keV).

scales although the statistical precision of our data hampers our ability to observe fluctuations less than about 5 counts s⁻¹ on time scales shorter than a day.

We were able to fit parameters describing the spectrum of MX1746–20 for the observations of January 1972. Because of the small number of data points available, however, we were unable to determine the nature of the spectrum unambiguously. For a trial thermal bremsstrahlung spectrum, a temperature of 7.7 ± 3.5 keV and a hydrogen column density of $1.1^{+1.0}_{-0.8} \times 10^{22}$ atoms cm⁻² were determined. For a power law spectrum, we found a photon spectral index of $2.1^{+0.8}_{-0.5}$ and a hydrogen column density of $5.3^{+3.0}_{-2.0} \times 10^{22}$ atom cm⁻².

A search of star catalogues and catalogues of interesting objects yielded only one likely candidate in the MX1746–20 error circle—the globular cluster NGC6440. NGC6440 is by far the most prominent object in the error circle and the only object brighter than $m_v = 10$, in spite of the high density of stars in the region. Using an empirical formula³ for the density

least two of the sources, MX0513–40 and MX1746–20, vary so greatly that they frequently fall below the level of detectability of the MIT OSO-7 instrument. We suggest that current and future satellite experiments, particularly those with high sensitivity, observe concentrated globular clusters from time to time since it is possible that a large fraction of them emit X rays on occasion.

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Abell 478 and X-ray source 3U0405+10

THREE sightings of the faint X-ray source 3U0405+10 with the Ariel V sky survey instrument have produced an improved position, with the error box now containing Abell 478. This distance class 6 Abell cluster is the most distant X-ray source yet detected. It has a (2–20 keV) X-ray luminosity of $(5.4 \pm 1.1) \times 10^{45} \text{ erg s}^{-1}$.

The instrument detected X-ray emission from 3U0405+10 on October 28, 1974, with both high (2.4–19.8 keV) and low (1.2–5.8 keV) energy detector systems. Combining these two lines of position with a later cross scan, from the HE system on March 17, 1975, the revised position of 3U0405+10 has been derived; correcting the Uhuru count rate to the new position gives a slightly higher rate statistically consistent with the Ariel V results.

The sky survey experiment scans a strip of sky around the spin plane of the satellite. The response of the collimators is designed to produce long, thin lines of position for the sources detected. The high and low energy systems are canted by $\pm 25^\circ$ respectively to the spin axis direction so that a source seen in both systems will have an error box limited to the crossing of the two lines of position. The FWHM of the collimators is 0.73° in the spin plane and 10.6° along the line of position. The width of each line of position has been taken as the FWHM of the collimator divided by the significance of the signal above the background. This corresponds approximately to a 90% confidence that the source lies within the boundaries of any line. Table 1 lists the observations and Fig. 1 shows the new error box, together with that from the earlier Uhuru observations.

The refined error box now contains the cluster of galaxies Abell 478 (Fig. 2) which was just outside the 3U error box¹. Furthermore, the area of the error box is now reduced to 0.27 square degrees from our data alone; combined with the

Fig. 1 Three intersecting lines of position from presently reported Ariel V observations give error box shown by thick line. Also shown are previous Uhuru position of 3U0405+10 and centre of A478 (1950 coordinates).

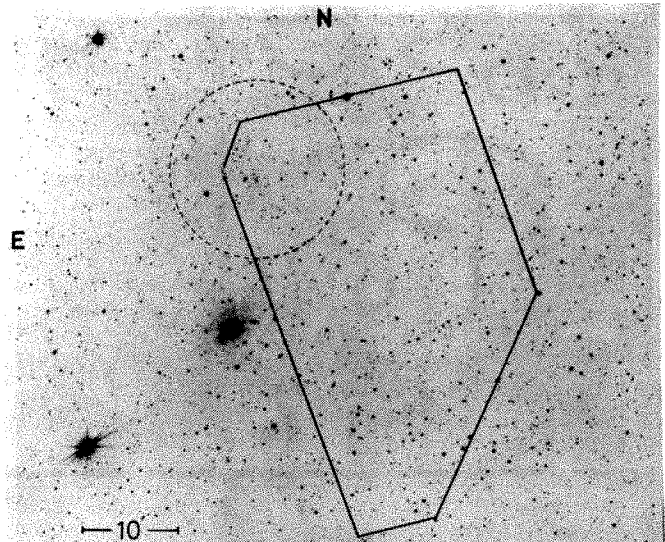
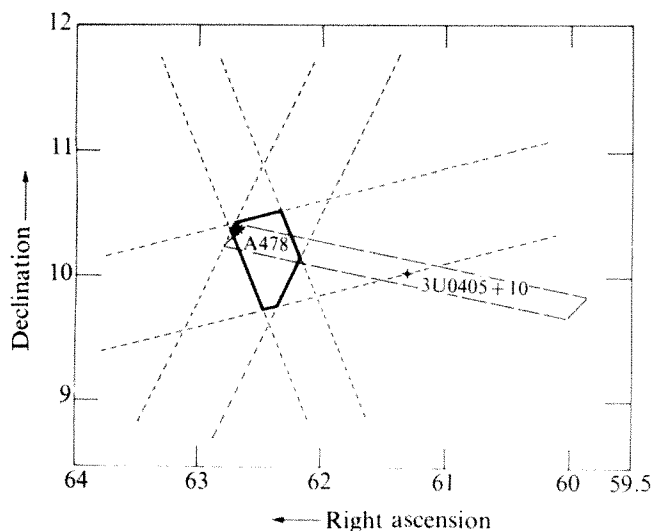


Fig. 2 Ariel V error box superimposed on Palomar Sky Survey photograph. The brightest galaxy in the A478 cluster is clearly seen in the north-east corner of the box; the circle indicates the approximate extent of the cluster. (Photograph copyright National Geographic Society–Palomar Observatory Sky Survey.)

Uhuru observations this is further reduced to ~ 0.1 square degrees. We regard this improved location as confirming the association of A478 with the X-ray source 3U0405+10. From a positional correlation of all the rich clusters in the Abell catalogue with the actual 3U sources and with similar distributions of sources from 20 'fake' catalogues, J. N. Bahcall and N. A. Bahcall (see ref. 2) have concluded that there is no good evidence for the association of any of the most distant (distance class 5 or 6) Abell clusters with X-ray sources in the 3U catalogue. Specifically, their analysis for 3U sources with error boxes ≤ 5 square degrees produced four associations with distance class 5 and 6, whereas the 'fake' source catalogue gave 2.7 ± 1.5 associations. The fivefold reduction in the error box area of 3U0405+10 greatly increases the probability of a physical association with A478, a rough estimate based on the number density of clusters and of 3U sources now giving a probability in excess of 80%.

Taking Abell's redshift value of 0.18 for distance class 6 clusters³ and a Hubble constant of $60 \text{ km s}^{-1} \text{ Mpc}^{-1}$ gives a distance of 900 Mpc. If we take the mean energy of our measured flux to be 4.5 keV (as it would be for a spectrum like that of the Crab source) then the two high energy measurements, combined, give an X-ray luminosity (2.4–19.8 keV) of $(5.4 \pm 1.1) \times 10^{45} \text{ erg s}^{-1}$. This makes A478 the most distant X-ray source

Table 1 Observations of 3U0405+10 with Ariel V sky survey

Date	Energy band (keV)	No. of orbits summed	Incident flux* ($\text{erg cm}^{-2} \text{ s}^{-1}$)
October 28, 1974	2.4–19.8	18	$(4.6 \pm 1.2) \times 10^{-11}$
October 28, 1974	1.2–5.8	18	$(6.6 \pm 2.3) \times 10^{-11}$
March 17, 1975	2.4–19.8	27	$(6.9 \pm 2.8) \times 10^{-11}$

*Assuming spectrum like that of Crab source.

yet detected and also the most luminous of all the X-ray cluster sources. Interest in the apparent relationship between the X-ray luminosity of clusters and the velocity dispersion of their brighter individual galaxies (for example, ref. 4) makes it of considerable importance to measure the value for A478, which is inferred, to lie in the range $4,000\text{--}7,000 \text{ km s}^{-1}$.

We thank Drs J. Bahcall and N. Bahcall for making available the results of their positional correlation studies, Dr M. V. Penston

for providing the overlay of Fig. 2 and the Hale Observatories for permission to reproduce the Palomar chart.

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Galactic X-ray sources and the ionospheric D region

SEVERAL authors (see refs 1-5 for example) have studied the possible influence of galactic point X-ray sources in the ionospheric D region. The observed variabilities in phase and amplitude of very low frequency (v.l.f.) night-time propagation have been attributed to different ionisation sources, but the relationship between the appearance of strong X-ray sources in the sky and the recorded v.l.f. phase and intensity variations has been emphasised. Particularly important are the effects of Sco X-1, Cen X-2 and Cen X-4.

Ion production rates attributable to galactic point X-ray sources have been evaluated², considering a constant photoionisation equal to $1/0.032 \text{ keV}^{-1}$ and energies of up to 10 keV. These calculations do not include all of the energy ranges available in the measured spectra. Therefore, the results obtained suggest that the contribution of point X-ray sources to the total ion production of the D region is small.

We calculate here theoretical ionisation rates which include energies up to 50 keV, non-constant photoionisation yields⁶ and the latest available measured spectra. The total electron production rate attributable to a celestial X-ray source is given by:

$$Q(z) = \int_{E_0}^{E_{\text{max}}} q(E, z) dE$$

where

$$q(E, z) = I(E, z) \sum_i \gamma_i(E) \sigma_i(E) n_i(z)$$

and

$$I(E, z) = I_0(E) \exp[\sec \theta \sum_i \sigma_i(E) \int_z^{\infty} n_i(z) dz]$$

where, $I_0(E)$ is the intensity of the emitted X rays at the top of the atmosphere; suffix i indicates the neutral constituent species considered (N_2 , O_2 and O); $n_i(z)$ is the number density of neutral atmospheric constituent i at altitude z ; $\sigma_i(E)$ the absorption cross section of neutral atmospheric constituent i for electromagnetic radiation over an energy range E ; and $\gamma(E)$ is the photoionisation yield, that is, the number of electron ion pairs formed per photon with energy E absorbed by a neutral atmospheric constituent i .

Figure 1 shows the ionisation rate attributable to Tau X-1 (ref. 7), Sco X-1 at 0° , 30° and 45° for normal conditions⁸ where $I_0(E) = 110 \exp(E/4.3)$, and for flare conditions⁹ together with the ion production attributable to other minor point X-ray sources in the southern sky¹⁰. It is important to

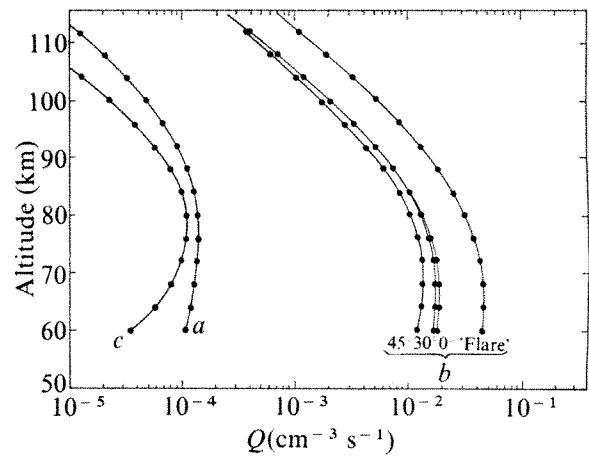


Fig. 1 Rates of ionisation production from: a, Tau X-1; b, Sco X-1; c, other minor sources in the Southern Hemisphere sky.

note that the profiles shown differ considerably from those given in the publications mentioned.

Figure 2 shows the ionisation rates attributable to major night-time ionisation sources: Lyman β , considering the flux given by Young *et al.*¹¹; scattered Lyman α calculated using the NO density distribution given by Meira¹², and the flux given by Winter *et al.*¹³; cosmic rays¹⁴ and the contribution of a diffuse galactic X-ray background⁶.

The contribution of Sco X-1 to the night-time ion production, is significant compared with the other major night-time sources, but the effect of the minor X-ray sources included can be neglected. Therefore, these results support claims that there is a Scorpius X-1 effect on the v.l.f. signal propagation records.

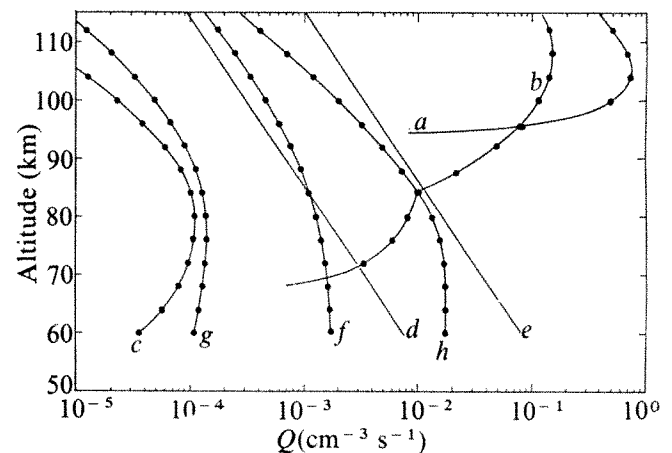


Fig. 2 Rates of ionisation production from major night-time ionisation sources: a, Lyman- β radiation; b, scattered Lyman- α radiation; c, stellar X-ray sources; d, galactic cosmic rays ($\gamma_m = 0^\circ$); e, galactic cosmic rays ($\gamma_m = 55^\circ$); f, diffuse galactic X-ray background; g, Tau X-1; h, Sco X-1.

Similar conclusions could be drawn for Cen X-2 (ref. 15) and Cen X-4 (ref. 16) since it has been shown that their spectral shape and intensity are comparable with those of Sco X-1.

This work has been carried out under the direction of Professor Sandro Radicella at the Aeronautics Department, Universidad Nacional de la Plata.

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Geomagnetic effect associated with X-ray flare from Sco X-1

THE effect on the night-time lower ionosphere of X radiation from celestial sources is a topic of some controversy¹⁻⁴. An X-ray flare from Sco X-1 has been shown to have a daytime effect on the ionosphere⁵ and it has also been reported⁶ that celestial X-ray sources have a nocturnal effect on the lower ionosphere in certain conditions. Galactic X-ray ionisation has been shown⁷⁻⁹ to affect night-time geomagnetic field components.

We have examined the possibility that an X-ray flare from Sco X-1, detected in the course of a balloon flight on October 15, 1967 at Mildura (34° 12'S; 142° 6'E)¹⁰, had a daytime geomagnetic effect. We observed a conspicuous perturbation in the form of an increase in the horizontal component of the magnetic field observed at the equatorial station at Kodaikanal (10° 14'N, 77° 28'E).

On October 15, 1967, Sco X-1 flared up at about 0700 UT, reaching a maximum in less than 10 min and then decreasing during the next 20 min (Fig. 1). The perturbation in the horizontal component of the magnetic field started around 0715 UT, and rose to a maximum at 0725 UT (an increase in the *H* component of about 14 γ), about 16 min after the flare had reached a maximum at around 0710 UT. Unfortunately, a small optical solar flare occurred about 15 min before the Sco X-1 flare, during the period 0647-0710 UT, with a maximum at 0650 UT.

In view of this, we have examined the possibility that the observed geomagnetic effect was caused by this solar flare, using the available solar X-ray flux data for this event. The procedure followed is similar to that adopted by Ramanamurthy et al.⁵. Solar X-ray flux data show a small burst in the 8-12 Å band during the period 0640-0713 UT but it is considered unlikely that it produced a solar flare effect (SFE), (geomagnetic crochet) as its amplitude is below the threshold value. Van Allen's data from Explorer 35 show a flare (with a flux enhancement of 2.3) which began at about 0640 UT, reached a maximum at about 0653 UT and ended at about 0730 UT. An earlier study made by us showed that the relaxation time of the SFE (geomagnetic crochet) with reference to the solar X-ray flare (1-20 Å) ranges from -10 to +22 min (with an average value of 4.0 min for the 1-8 Å band and 1.4 min for the 8-20 Å band). Thus, the observed time lag of about 33 min between the solar X-ray flare (2-12 Å) maximum and that of the observed geomagnetic effect is well outside the range of relaxation times for geomagnetic solar flare effects (SFE or crochet events).

The possible daytime geomagnetic effect of Sco X-1 is considered more interesting than the nocturnal effect reported earlier, as during the day the rate of ion production resulting from the Sco X-1 X-ray flux has to compete with the rate resulting from the solar X-ray flux and L_{α} besides that resulting from galactic cosmic rays. An estimation of the production rates

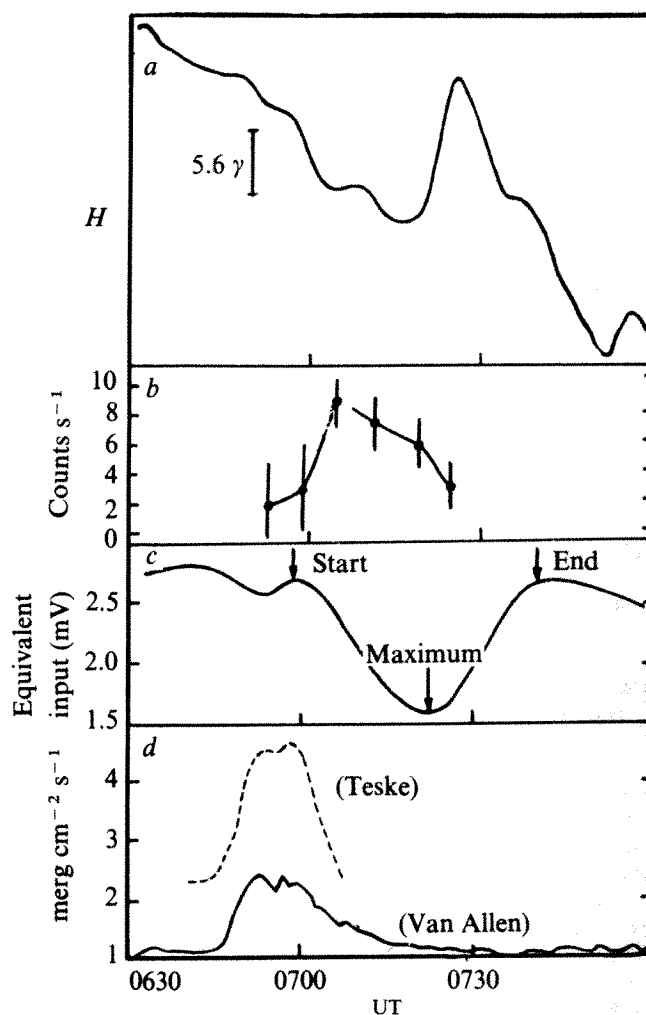


Fig. 1 Kodaikanal, October 15, 1967; temporal variations in: a, the horizontal component, *H* of the magnetic field at Kodaikanal; b, 20-30 keV X-ray counts from Sco XR-1 (ref. 10); c, the field strength (164 kHz) over the Tashkent-Delhi path (after Ramanamurthy et al.⁵); d, the solar X-ray flux 2-12 Å (Van Allen) and 8-12 Å (Teske), (after Ramanamurthy et al.⁵).

attributable to Sco X-1, L_{α} and galactic cosmic rays indicates that the rates of production attributable to the X-ray source are comparable with those attributable to cosmic rays and L_{α} at levels below about 70 km (ref. 5). So the observed geomagnetic effect could be attributable to X-ray flux enhancement (1-20 Å), as during an SFE (crochet) the region of enhanced conductivity lies in the D-region¹¹⁻¹⁴.

A sudden impulse (SI) was reported on October 15, 1967 at 0724 UT from two stations, SO and HL and an SSC was reported from station SU (Solar-Geophysical Data ESSA/NOAA). In view of the circumstantial evidence presented here, however, we do not consider the observed geomagnetic perturbation at Kodaikanal as an SI or an SSC but as a genuine effect of the X-ray flare from Sco X-1.

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Ghost echoes on the Earth–Moon path

ON July 7, 1974 while using a Moon Bounce technique on 1,296 MHz I observed the appearance of strange, delayed echoes. My equipment consists of a parabolic antenna 26 feet in diameter with a circularly polarised feed horn driven with 500-W continuous wave from a transmitter. The receiver has a noise figure of 2 dB and a bandpass of 500 cycles and the equipment had a very distinct note because of a spurious frequency near the fundamental; on the Moon–Earth circuit it is very easy to identify this signal because of this unique characteristic. On the day in question a series of dots or a single dash were being reflected back from the Moon after 2.6 s. Suddenly there appeared a second signal delayed by approximately 2 s. This signal had the same characteristics of the Moon Bounce signal except that it was weaker.

At the time of the observations it was afternoon, the Sun was almost due west and the Moon was to the south-west with an altitude of about 30°. Throughout a series of transmissions the returning Moon signal was followed about 2 s later by the delayed ghost signal with the same characteristic note of the transmitter. Unfortunately, I could not record the signals though they continued for 20 min. When I failed to track the Moon with my antenna, the Moon signal would fade but the echo remained at about the same strength.

The following day a severe radio blackout occurred, and lasted for several days, coincidentally with the appearance of a large sunspot. Relating my reception of the ghost echo with this violent solar eruption I suggest that the large streamer of gas from the corona of the Sun produced a highly ionised cloud which reflected the radio signals I had directed towards the Moon. If this streamer approached with a speed of 1,000 km s⁻¹, with a front like a shock wave, it could have acted as a good reflector. The cloud would have been about 800,000 km out in space, as indicated from the delay times of 4–5 s.

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²²Na, Ne–E, extinct radioactive anomalies and unsupported ⁴⁰Ar

A NEW picture¹ of the origin of the known extinct radioactivities (¹²⁹I and ²⁴⁴Pu) holds that these radioactive species were precipitated in grains forming in the rapidly cooling ejecta of explosive nucleosynthesis, and that their decay occurred in interstellar grains rather than in the meteorites. If so, our interpretation of extinct radioactivities is enlarged. Their detectability is no longer related to the usual criterion that they live long enough for the meteorites to form, but rather that they live long enough for grains to form in the expanding envelope.

My first point is to interpret ²²Na as a detectable extinct radioactivity. Its half life (2.6 yr) seems long enough for an expanding gas to cool to the point of grain formation. If we take the adiabatic relationship $p/T^3 = \text{constant}$ for purposes of a simple estimate, we find that matter having a density of

10⁴ g cm⁻³ at $T=10^9$ K has a density of 10⁻¹⁴ g cm⁻³ when $T=10^3$ K where grains can form copiously. The observations² of Nova Serpentis 1970 confirm very extensive grain formation in similar circumstances on a time scale of a few days. If the expansion speed of a supernova envelope is 10⁴ km s⁻¹, this drop in density requires about 3 yr. Condensation temperatures may be reached even earlier if the expansion is not adiabatic, but also radiates.

The ejection of ²²Na in large amounts is expected from the helium shells of explosive supernovae³ and from the surface explosions of novae⁴, both at the level 10⁻³ g g⁻¹. I have studied these for their prospects for nuclear gamma-ray astronomy, but since ²²Na may in part be expected to precipitate in grains before it decays, we may also expect the existence of interstellar grains being isotopically rich in ²²Ne in comparison with the Sun. The admixing of these grains without vaporisation into the forming carbonaceous chondrites is a good candidate for the source of the ²²Ne-rich neon component Ne–E, which was discussed by Black⁵, who attributed it to an extrasolar source. It is at least as ²²Ne-rich as ²⁰Ne/²²Ne ≤ 3.4, and could conceivably be pure ²²Ne. The fact that the released gas is purest in Ne–E around 1,000 °C in stepwise heating probably reflects the fact that ²²Ne occupies a refractory ²²Na site in the grains. The absolute amounts of ²²Ne are also relatively constant near 10⁻⁹ cm³ g⁻¹ STP, suggesting uniform sprinkling of presolar grains through C1 chondrites.

The ²²Na yield is more than adequate to account for the Ne–E anomaly. Nucleosynthesis calculations suggest, but do not prove, that the fraction of all ²²Ne that was synthesised as ²²Na, in specific ejecta concentrations near 10⁻³ g g⁻¹ (refs 3, 4), is about 10⁻², which is also about 5 × 10⁻² of the total stable ²³Na abundance. This total nucleosynthesis yield is quite large, and about one-tenth of it is here supposed to be incorporated into grains before the ²²Na decay. I have argued¹ that the fraction f_{Na} of condensed solar system Na that existed as unvaporised presolar grains lies between 10⁻¹ and 10⁻². If these estimates are correct, the concentration of Ne–E potentially available if neon gas were not lost from grains is between 5 × 10⁻⁴ and 5 × 10⁻⁵ of stable Na. That this is many orders of magnitude greater than observed Ne–E concentrations reflects the loss of daughter neon from the hot but unvaporised grains.

A correlation of Ne–E with Na concentration would not necessarily be expected, however, because these same explosively ejected zones are not rich in stable ²³Na. This Ne–E is a component of Ne–A (ref. 5), which shares a “threshold-for-appearance” effect with He–A, which is deficient in ³He in comparison with the solar wind. Component A is interpreted by Black⁵ as a linear combination of E and a component D, which he interprets as a primitive solar wind, before the deuterium has burned to ³He. In the framework of the present discussion, we see that D may also contain the dense gas surrounding the newly formed grains in the expanding supernova. I suggest interpreting E as ²²Ne from ²²Na decay and essentially pure ⁴He from the helium shell in which the ²²Na is synthesised³. Other isotopes of neon, especially ²¹Ne, also exist in the gas, but $(^4\text{He}/\text{Ne})_{\text{gas}} \approx 10^4$ so that ⁴He should be the dominant trapped gas. Only if neon is trapped more efficiently than helium in this environment will the newly synthesised neon gas contribute a component to E as well. Calculations of this neon synthesis have been published^{6,7}. Arnould and Beelen⁷ noted the similarity to Ne–E of special cases of the total gas yield, but the ²²Na interpretation seems more plausible since it is much more easily trapped than neon gas.

Black⁵ also calls attention to the fact that in the C2 chondrite Nagoya the dark gas-rich phases contain 3 times as much ⁴⁰Ar as the light gas-poor phases, although the light phases have a higher fraction of Ne–E in their total neon. Perhaps the explanation is that the dark phases contain a higher fraction of presolar grains. The ⁴⁰K/⁴¹K ratio was initially much higher in nucleosynthesis events, so grains forming there rather than in the solar system would now have more ⁴⁰Ar/⁴¹K. This suggestion is not necessarily inconsistent with the bulk ²⁰Ne/²²Ne

ratios, because the dark gas-rich portions may contain absolutely more Ne-E than the light phases. It has simply been diluted by more captured gas. In any case, it must be pointed out that such grains would seem to have "unsupported ^{40}Ar ". Interstellar grains accreted by the lunar surface, as I have suggested¹ from unsupported fission xenon, would also show unsupported ^{40}Ar . Since unsupported ^{40}Ar has been found in lunar soil⁹, this idea as a partial source should not be overlooked.

The views expressed here suggest many correlations to look for. Calcium should be a rich element for anomalies. The greatest may be at ^{44}Ca , which we⁸ have shown is synthesised as 47-yr ^{44}Ti . Titanium is also refractory, so both elements condense out early, but a $^{44}\text{Ca}/^{40}\text{Ca}$ correlation with Ti/Ca is here predicted in the small scale structure. The heaviest isotopes of Ca are, moreover, probably synthesised¹⁰ in different mass shells from ^{40}Ca , so they may also be expected to show small scale anomalies. Since ^{41}K is synthesised as radioactive ^{41}Ca (ref. 8), small ^{41}K excesses correlated with calcium concentration are predicted. Anomalies due to ^{26}Al should also be interpreted in terms of presolar grains rather than a primordial concentration.

Our calculations⁸ show that ^{53}Cr is synthesised as parent ^{53}Fe , which is arrested at 2×10^6 yr ^{53}Mn . In grain formation, therefore, a substantial part of this yield will chemically correlate with Mn. That is, a correlation of $^{53}\text{Cr}/^{52}\text{Cr}$ with Mn/Cr is predicted. Peculiarities at ^{54}Cr are also expected, in as much as it is synthesised in a separate process¹¹.

Another outstanding candidate is iron: ^{54}Fe is synthesised¹¹ along with $^{56,57}\text{Ni}$, which are themselves held up for months to years before their transmutation to $^{56,57}\text{Fe}$ is complete. Grains forming in this period could show Ni or Co correlated anomalies due to chemical effects. They will also be deficient in ^{56}Fe , which is synthesised elsewhere¹¹. Other possibilities suggest themselves once the key idea is in mind.

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Preferential orientation in four C3 chondritic meteorites

It is more than a century since oriented fabrics were first noted in chondrites (see ref. 1), but since then little work has been done on the topic^{2,3}, the most extensive being that by Dodd⁴. He found that most of his H- and L-group chondrites possessed foliation, together with lineation in the most strongly foliated specimens. The preferential orientation of elongated olivine phenocrysts in porphyritic chondrules has also been observed⁵.

A visual examination of the Leoville C3 chondrite disclosed a strong orientation of the long axes of the chondrules. This prompted us to compare the orientation of the long axis of the chondrites in this meteorite with three other Type 3 carbonaceous chondrites: Grosnaja, Coolidge and Vigarano. We were unable to obtain specimens cut with three mutually perpendicular faces, the most desirable configuration for a study of this kind. Our preliminary samples generally possessed only one face cut

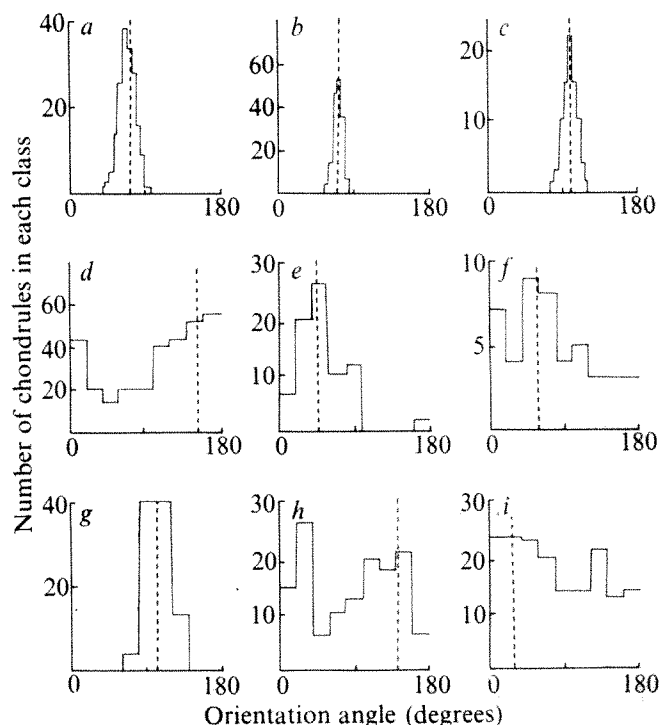


Fig. 1 Histograms of the orientations of the long axes of chondrules in four C3 carbonaceous chondrites. Leoville specimens: a (LU 44484), vector magnitude (V_m) = 94.1%; b (BM 1969, 144), V_m = 98.5%; c (LU 44484), V_m = 95.6%. Three faces of Vigarano specimen (BM 1924, 15); d, V_m = 29.3%; e, V_m = 67.8%; f, V_m = 24.1%. Grosnaja specimen (BM 63624): g, V_m = 86.1%; h, V_m = 14.1%; i, V_m = 11.2%. Marked lines, mean vector directions.

at random, and we may, therefore, have encountered some of the problems outlined by Dodd⁴.

We thus decided to photograph each available face using fine-grained monochrome film, and then to prepare appropriate enlargements. This avoided the problem posed by the removal and transport of actual specimens and facilitated magnification to a suitable size. Individual photographs were mounted on the table of an orientometer designed to allow movement in translation and rotation⁶, and were viewed through a binocular microscope fitted with a line reticule in one eyepiece. Chondrules were aligned with their long axis along this fiducial line, and their nominal orientation was read from the degree scale of the orientometer turntable. Each measured image was marked with a spot of ink to avoid confusion and possible duplication. The measurements are of the double-headed vector type⁷, so only the direction between 0° and 180° was taken.

Chondrule orientations for each specimen were grouped into 5°, 10° or 20° classes according to the number of measurements and their scatter, and the resulting histograms are shown in Fig. 1. The mean vector direction and its magnitude are also shown, complete orientation being expressed as 100% and a random distribution by 0% (ref. 7).

Histograms give a good visual idea of the presence and degree of any orientation and our measurements indicate a high degree of orientation of the long axes of chondrules from all specimens of the Leoville chondrite, in the Grosnaja specimen, and on face b of the Vigarano specimen (Fig. 1 a, b, c, e and g). The other two faces of Vigarano (Fig. 1 d and f) exhibit a much lower degree of chondrule orientation, and the Coolidge specimens (Fig. 1 h and i) show a wide scatter. These qualitative impressions are borne out by the calculated vector magnitude.

From these results it seems that the orientations of the long axes of chondrites are strong in Leoville and Grosnaja chondrites, moderate in one plane of the Vigarano specimen, and very weak or nonexistent in the Coolidge specimens. Dodd⁴ could find no preferred orientation in the last-named

meteorite. The wide range of degrees of orientation between the four C3 chondrites examined also supports Dodd's conclusion⁴ that there is no correlation between orientation and degree of metamorphism.

The identification of the mechanisms which produced asphericity and alignment of the chondrules in some chondrites is not an easy matter. Are these phenomena related to the primary chondrule-forming process, to effects occurring during aggregation, or are they a result of secondary processes in the meteorite parent body? Are the two phenomena related? More data are needed before these questions can be resolved.

For this reason we are at present examining the orientations of the long axes of chondrules in more detail, along with such factors as shape and size distribution, density, friability, and so on. It is hoped that these studies will enable us to draw conclusions concerning the formation of chondrules and the genesis of meteorites.

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Possible ozone depletions following nuclear explosions

HAMPSON¹ has commented on the possible destruction of the Earth's ozone shield by nitric oxide generated during nuclear explosions in the atmosphere. He suggested that if such an ozone decrease were large enough and lasted long enough, it might have disastrous effects on the biosphere. Here, we assess quantitatively Hampson's suggestion.

We have considered only nuclear detonations occurring near the ground, with the NO formed and carried into the stratosphere by rising fireballs. High altitude bursts would probably be much lower in total yield; moreover, NO injected above the stratosphere has a shorter photochemical lifetime and is less effective in removing ozone than is NO deposited in the stratosphere. To calculate the quantity and distribution of NO in the atmosphere after a series of large nuclear explosions, we have assumed that each megaton of nuclear yield generates 10^{32} molecules of NO, an average value between upper and lower limits^{2,3}. We have assumed that the NO is uniformly mixed in the nuclear cloud at stabilisation, when it has a spheroidal shape and lower and upper altitudes given by the empirical curves of Peterson⁴ for 30° to 90° latitudes.

For the total yield we have chosen two values: 5,000 Mton (case 1), as suggested by Hampson¹, and twice that, 10,000 Mton (case 2), in order to assess a more severe case. In both cases the total yield was provided equally by 1 Mton and 5 Mton devices. This choice is approximately consistent with published tabulations of strategic nuclear armaments⁵ delivered by missile. To determine the sensitivity of the results to the individual yields, an additional case consisting of 5,000 Mton provided equally by 1 Mton and 3 Mton devices, has also been treated. The injected NO is assumed to be uniformly distributed either between 30° and 70°N, or over the entire Northern Hemisphere. The latter

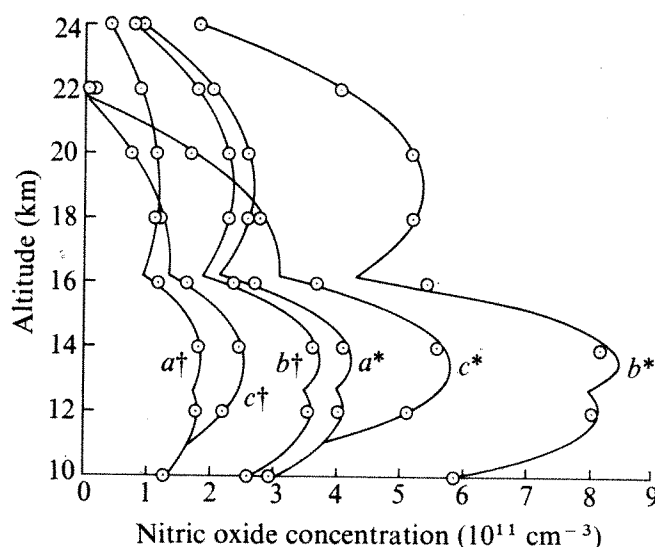


Fig. 1 Initial concentrations of nitric oxide generated by nuclear explosions for total yields of: a, 5,000 Mton produced equally by 1 and 5 Mton devices; b, 10,000 Mton produced equally by 1 and 5 Mton devices; c, 5,000 Mton provided equally by 1 and 3 Mton devices. The NO molecules are assumed to be uniformly spread over: *, zone between 30° and 70°N; †, the Northern Hemisphere. Each circle point represents an average concentration for a 2-km vertical interval (the model grid spacing) centred at that altitude; that is, it is an average concentration in each 2-km segment.

case is included because hemispherical dispersion can occur in a time (several months) which is short compared with the stratospheric residence time of the NO (several years). The 'initial' NO concentrations for each case are shown as functions of altitude in Fig. 1.

If the nuclear bursts actually were confined between 75°–125°W and 25°–75°E, corresponding roughly to the geographical boundaries of the USA and the populated USSR, it would take about as long for the NO to spread uniformly over the area between 30° and 70°N (ref. 6), as it would take for the ozone column density to reach a minimum (about 2 months). Accordingly, our calculations using longitudinally averaged NO concentrations are only estimates of the average ozone reductions to be expected. A higher initial concentration of the NO_x generated by fireballs in certain geographical regions ensures that the ozone reduction will be somewhat larger in those areas.

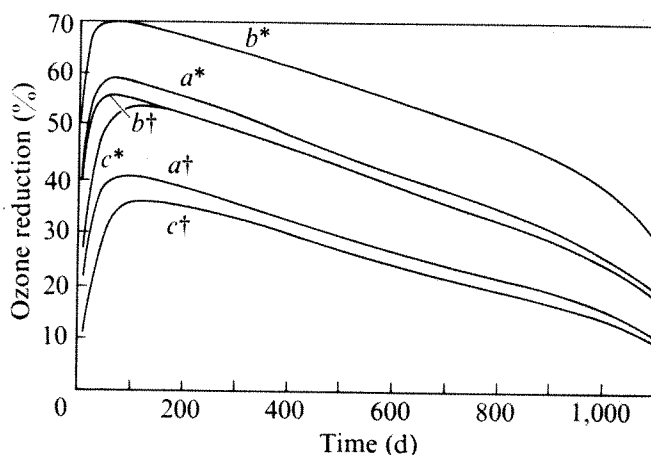


Fig. 2 Depletion of the total ozone column as a function of time after a series of large nuclear explosions for cases described in Fig. 1.

The depletion of ozone and atomic oxygen by catalytic reaction with nitrogen oxides produced by nuclear explosions ($O_3 + NO \rightarrow O_2 + NO_2$; $O + NO_2 \rightarrow O_2 + NO$) was assessed using a one-dimensional numerical model of stratospheric and mesospheric constituents that includes 150 photochemical reactions (see ref. 7). The model predicts the concentrations of 46 chemical constituents; only the species O, O(¹D), O₃, OH, HO₂, NO, NO₂ and HNO₃ need to be considered here. The vertical transport of stratospheric gases is represented by an eddy diffusion process; we have selected a diffusion coefficient⁸ which reproduces the vertical distribution of methane at altitudes above 30 km and also gives the proper stratospheric residence times for radioactive tracers (H. S. Johnston, P. Kattenhorn, and G. Whitten, unpublished). This model, however, has a tropopause level at an altitude of 16 km, which is appropriate only for low latitudes; between 30° and 70° the average tropopause is near 11 km. Accordingly, we have shifted the Wofsy and McElroy⁸ diffusivity curve downward by 5 km. The predicted ozone reductions are, of course, sensitive to the eddy diffusivity profile used. In view of the constraints placed on vertical turbulent transport rates by the tracer data, we believe that uncertainties in the calculated ozone depletions resulting from uncertainties in the eddy diffusion coefficients are much less than a factor of two, and are probably not greater than 25%. Our calculations were performed with the Sun at a fixed elevation of 45°. It is valid⁹ to assume a fixed rather than a diurnally varying solar zenith angle when predicting the effects on ozone of artificially injected NO_x.

Figure 2 shows our computed ozone column depletions as a function of time after a large-scale nuclear detonation for combinations of yields and subsequent horizontal spreading. In cases *a* and *b* the peak ozone reductions occur after about 2 months, with large depletions persisting for several years. When the lower weapon yields of 1 Mton and 3 Mton are assumed (case *c*), the NO is injected lower in the stratosphere (see Fig. 1) and our results show slightly smaller ozone reductions for the same total yield as in case *a*, and about twice as long a delay to the peak ozone reduction. The *e*-folding time for ozone recovery is about 3 yr in our model, reflecting the slow transfer of NO_x compounds from the stratosphere to the troposphere by eddy diffusion. After about a year the NO_x should be spread fairly widely throughout the northern hemisphere⁶, thus reducing its concentration to about half the value it would have had were it still confined to the 30°–70°N zone. Comparison of the *a*[†], *b*[†] and *c*[†] curves in Fig. 2 with the corresponding * curves shows that this spreading decreases the ozone reduction by about 20–30%. In the former cases, however, the ozone reduction occurs over the entire Northern Hemisphere, not just in the zone between 30° and 70°N.

Figure 3 shows the post-detonation variation of the ozone concentrations at several altitudes for case *b** in Fig. 7; that is, NO produced by 10,000 Mton distributed between 30° and 70°N. Below the tropopause (~11 km altitude), excess NO_x is so quickly removed by rainout, and its catalytic reaction cycle with ozone is so slow because of low atomic oxygen concentrations, that there is little ozone reduction. In the stratosphere up to about 22 km, the peak ozone reduction occurs more quickly with increasing height as a consequence of the increasing abundance of O. Most of the NO_x above 22 km has been transported upwards by diffusion from lower altitudes, so the peak ozone reduction occurs later for higher altitudes, reflecting the time required for upward transport of NO by eddy diffusion. Eventually, all of the excess stratospheric NO_x is transported either downward into the troposphere where it can be washed from the air by rainfall or upwards into the mesosphere where it can be photolysed into N and O and destroyed by the subsequent reaction $N + NO \rightarrow N_2 + O$.

Our calculated ozone depletions are only estimates because of the expected inhomogeneous distribution of NO produced

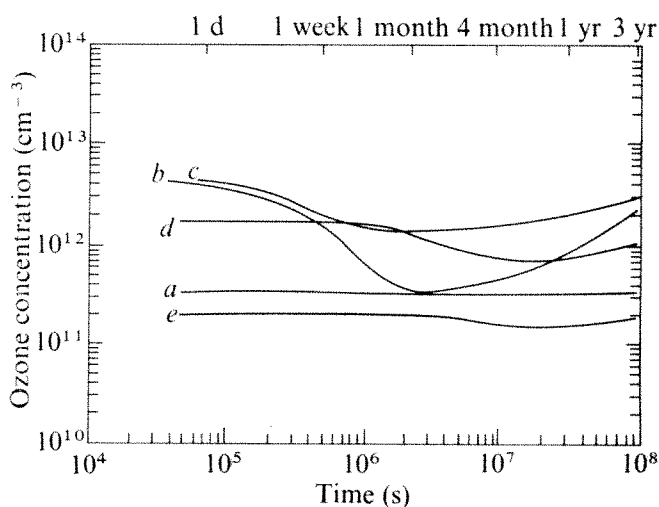


Fig. 3 Variations of the ozone concentrations over time at several altitudes for case *b** in Fig. 1. (10,000 Mton zonally distributed): *a*, 10 km altitude; *b*, 16 km altitude; *c*, 26 km altitude; *d*, 36 km altitude; *e*, 46 km altitude.

by nuclear explosion, because of uncertainties in the total amount of NO generated (perhaps a factor of 2) and its initial altitude distribution, and because of probable substantial changes in stratospheric circulation caused by very large ozone depletions. Moreover, our computations apply to mid-latitude conditions, that is, somewhere between 30° and 70°N. For more accurate calculations, a photochemical model with multidimensional transport including thermally-driven winds is required. We do not, however, expect the results from such a study to differ markedly from those reported here, and we therefore conclude that Hampson's concern is well founded.

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Trapa natans in the British Flandrian

POLLEN of *Trapa natans* L. the water chestnut, has been found in two Flandrian (Holocene) deposits at Skipsea, North Humberside, providing the first *in situ* Flandrian records of this species in Britain. The occurrence, followed by the extinction, of *T. natans* in Scandinavia has been regarded as evidence of a 'climatic optimum' when summer temperatures were at least 2 °C higher than at the present day¹⁻⁴. The new British data may indicate similar conditions but could also record human activity, in which case it would not be necessary to invoke climatic change.

We studied two former meres occupying depressions on the surface of the Devensian (Weichsel) till. The pollen was

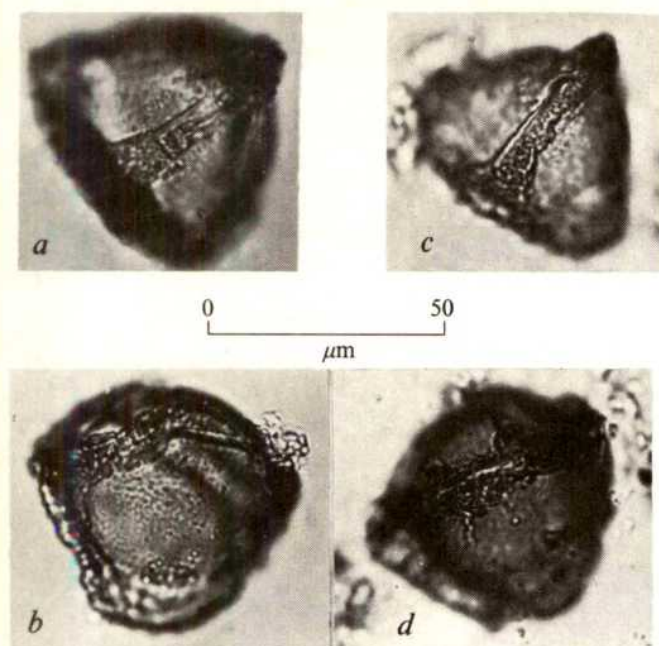


Fig. 1 Pollen grains of *Trapa natans* L. a, b, Reference pollen; c, fossil grain from 2.75 m, Skipsea Bail Mere; d, fossil grain from 2.87 m, Skipsea Low Mere.

found in the fine detritus mud of a 5.50-m core from the site of the former Skipsea Low Mere⁵ (os ref.: TA 155563) at Skipsea, near Hornsea, North Humberside. The total number of *Trapa* grains seen in pollen preparations of samples taken at 0.25 m intervals is shown in Table 1.

The pollen was also present in fine detritus mud of a 4.00-m core from the site of Skipsea Bail Mere⁵ (os ref.: TA 162552) nearby. Examination of prepared slides revealed single grains at 2.75 m, 2.90 m, and 3.25 m, but not in intermediate samples.

The pollen was determined as *Trapa* by comparison first with drawings⁶ and photographs⁷, and, subsequently, with reference material (Fig. 1). *T. natans* is the only species of Trapaceae found at present in western Europe.

There are at least four possible explanations of the find: laboratory contamination, derivation from interglacial deposits, introduction by birds, and actual occurrence of the

Table 1 Total number of *Trapa* grains seen in complete scanning of pollen preparations 0.25 m apart in a core from Skipsea Low Mere

Depth	Number of grains
2.12 m and above	0
2.37 m	1
2.62 m	2
2.87 m	12
3.12 m	1
3.37 m and below	0

species at the two meres in the Flandrian. Contamination seems very unlikely, since no specimen or sample of *T. natans* or of any deposit known to contain it had ever entered our laboratory previously. It is certainly possible that the grains are derived (indeed one specimen is somewhat eroded); for that to be correct, however, requires that the grains were derived twice: first from an interglacial deposit into the Devensian till which surrounds the meres, and then into the mere deposits. The samples contained no other pollen of types which are necessarily derived, nor did they have the appreciable content of mineral matter which could be expected if derivation from till had occurred. Transport of pollen by birds is possible. Seeds and fruits adhere to birds⁸, and pollen may travel in the same way. The nearby Hornsea Mere is an important staging post for waders migrating from Scandinavia (J. E. S. Walker, personal communication) at a time when *T. natans* is flowering (June–September³). Carriage by birds cannot, therefore, be excluded, but should be considered as a remote possibility in view of the regular appearance of the pollen at several stratigraphical levels. The presence of *Trapa* pollen probably indicates that the taxon was living at the site⁹.

The single previous record from the British Flandrian¹⁰ is of a fossil fruit found floating in a brackish loch in the Outer Hebrides. Doubt has been cast on this record⁴ because of the general unlikelihood that the species would occur in so oceanic an environment; in addition the fruit had peat (apparently of the *Sphagnum-Calluna* type) adhering to it, suggesting a bog habitat, whereas *T. natans* is a floating aquatic. Those objections do not, however, apply to the records reported here: North Humberside is not particularly oceanic and the pollen was found in fine detritus mud, representing an environment in which floating aquatics could have existed. The new records should be accepted, provisionally at least.

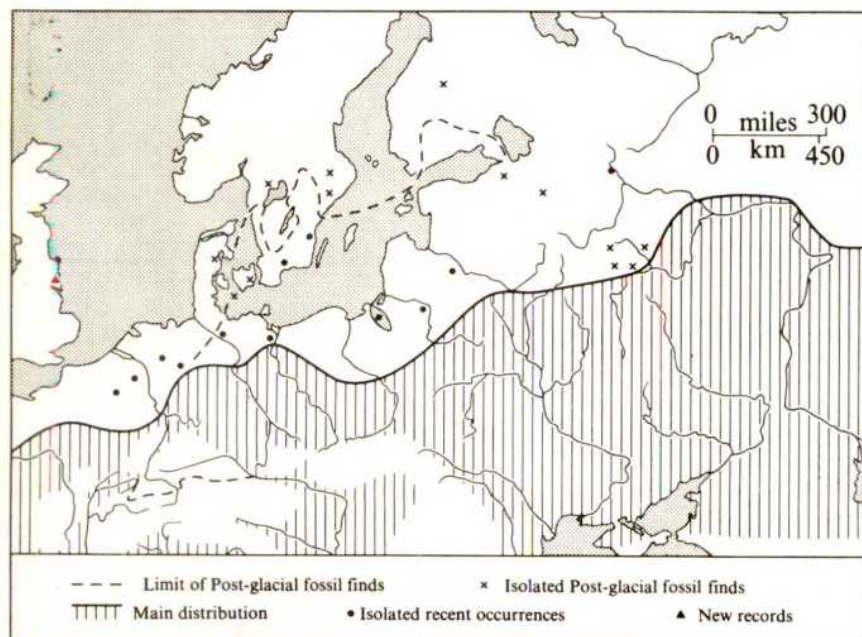


Fig. 2 Distribution of *Trapa natans* (partly after ref. 3).

A pollen and spore count from the main *Trapa* horizon, 2.87 m deep at Skipsea Low Mere, gave the results shown in Table 2.

Outline pollen diagrams showed that the other samples containing *Trapa* from the two meres give similar spectra. These results suggested that *T. natans* was growing in North Humberside after the decline of *Ulmus* but before the main lowland forest clearances (that is, about 2500–500 BC, the period of Bronze Age cultures in Britain). That is the time during which *T. natans* attained its maximum northward extension in Europe, reaching Finland and southern Sweden (Fig. 2)^{1,2}. The arrival of *Trapa* in Northern Europe is usually attributed to ordinary, if rather slow, migration in response to the warm, postglacial climate, although cultivation by man has also been suggested.

Table 2 Pollen and spore count from the main *Trapa* horizon, 2.87 m deep at Skipsea Low Mere

<i>Pinus</i>	1	<i>Corylus</i>	60	<i>Sparganium</i>	2
<i>Ulmus</i>	5	Gramineae	3	<i>Trapa</i>	1
<i>Quercus</i>	68	Cyperaceae	1	Polypodiaceae	3
<i>Tilia</i>	10	Caryophyllaceae	1	<i>Filicales</i>	1
<i>Alnus</i>	85	<i>Solanum</i>	1	Deteriorated grains	4
<i>Fraxinus</i>	8	<i>Nuphar</i>	1		
					Total pollen and spores 255

Suggested causes of the decline of *Trapa* include³ climatic deterioration, a decline in the trophic status of fresh waters, the extinction of beavers, the collection for food by man, the cessation of cultivation as a food plant, disease, and degeneration caused by inbreeding. Most authors^{1–4} prefer a climatic explanation and correlate the decline with the aftermath of the 'climatic optimum' when summers were at least 2 °C warmer than at present. The new records could be used to support this figure.

Explanations relating to man, however, deserve re-examination in the context of the new records. Water chestnuts have been an important source of food in Europe and elsewhere⁵. It is to be expected that a species on the edge of its range will be unusually sensitive to the depredations of man. The activity of a late Bronze Age culture in the area is confirmed by the presence of a substantial lake settlement in Skipsea Low Mere and another in the marshy area between Skipsea Low Mere and Skipsea Bail Mere¹¹. It seems likely that people living there would have taken advantage of this readily available food supply, and may thereby have caused its extinction. Alternatively, man could have introduced and cultivated water chestnuts. That would explain their arrival, and their extinction may have coincided with the decline of late Bronze Age culture.

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A new method of measuring the hydrogen bond stretching frequency ν_{σ} of a complex in solution

THERE is no agreed explanation of the broadening of the infrared absorption bands associated with the $\nu_{\sigma}(\text{XH})$ stretching mode of a hydrogen-bonded complex $\text{XH}\cdots\text{Y}$ in solution (see ref. 1). There is, however, a growing body of evidence to suggest that the distinctive appearance of these bands can be understood in terms of the anharmonic coupling of $\nu_{\sigma}(\text{XH})$ and $\nu_{\sigma}(\text{XH}\cdots\text{Y})$ modes^{2,3}, provided it is recognised that the phase coherence of the $\nu_{\sigma}(\text{XH}\cdots\text{Y})$ mode is rapidly lost owing to the strong interaction between the complex and the solvent⁴. The implication of this proviso is that the $\nu_{\sigma}(\text{XH}\cdots\text{Y})$ motion should be regarded as an oscillatory stochastic process rather than the oscillation of a conservative system.

A model developed by one of us (G.N.R., unpublished), which assumes an explicit form for the anharmonic coupling between the $\nu_{\sigma}(\text{XH})$ and $\nu_{\sigma}(\text{XH}\cdots\text{Y})$ modes and invokes the Ornstein–Uhlenbeck stochastic process to represent the hydrogen bond stretching vibration, allows a simple expression for the molecular dipole moment autocorrelation function to be obtained. This theoretical autocorrelation function depends on three parameters: (1) a mode-mode coupling constant; (2) a viscous damping coefficient, representing the effect of the solvent on the $\nu_{\sigma}(\text{XH}\cdots\text{Y})$ mode, and (3) the angular frequency of the hydrogen bond stretching vibration. By Fourier transformation of digitally recorded infrared spectra we have obtained experimental dipole moment autocorrelation functions for the phenol–acetonitrile complex in solution in carbon tetrachloride and for a number of other systems. Using the method of least squares we have been able to estimate numerical values and standard deviations for the three parameters of the model in each case. In particular, we have been able to measure the hydrogen bond stretching frequency with an accuracy of about 10%.

We believe that this provides the first absolutely conclusive evidence that the $\nu_{\sigma}(\text{XH})$ band profiles (Fig. 1) of hydrogen-bonded species in solution are indeed composite. Furthermore, our method of analysis provides a means of measuring the $\nu_{\sigma}(\text{XH}\cdots\text{Y})$ frequency in cases where the structure in the 3,000 cm^{-1} region of the spectrum is impossible to resolve visually and the far infrared spectrum cannot be recorded.

The theoretical result which is fundamental to the analysis is obtained by requiring that the displacement coordinate r_2 of the $\nu_{\sigma}(\text{XH}\cdots\text{Y})$ mode should obey the Langevin equation

$$\ddot{r}_2 + \beta \dot{r}_2 + \omega_2^2 r_2 = F(t) \quad (1)$$

If the random force $F(t)$ acting on the complex is supposed to be a Gaussian random variable with an infinitesimal correlation time, $r_2(t)$ is a representation of the Ornstein–Uhlenbeck stochastic process, the mathematical properties of which are well understood⁵. By assuming a coupling term in the molecular hamiltonian of the form $K_{112} r_1^2 r_2$ and treating the $\nu_{\sigma}(\text{XH})$ vibration as a quantum mechanical oscillator coupled to a classical stochastic oscillator, it is easy to obtain the dipole moment autocorrelation function

$$\Phi(t) = \exp \left\{ -i\omega_1 t - a^2 \langle r_2^2 \rangle \right. \\ \left. \times \int_0^t (t-x) e^{-\beta x/2} [\cos \tilde{\omega}_2 x + \frac{\beta}{2\tilde{\omega}_2} \sin \tilde{\omega}_2 x] dx \right\} \quad (2)$$

where $\hbar\omega_1$ is the $\nu_{\sigma}(\text{XH})$ energy quantum, $a = K_{112}/m_1\omega_1$, and $\tilde{\omega}_2 = (\omega_2^2 - \beta^2/4)^{1/2}$ in the underdamped case. A similar, but aperiodic, result holds in the overdamped case where $\beta > 2\omega_2$. It is worth noticing that β is determined⁶ by the variance of the

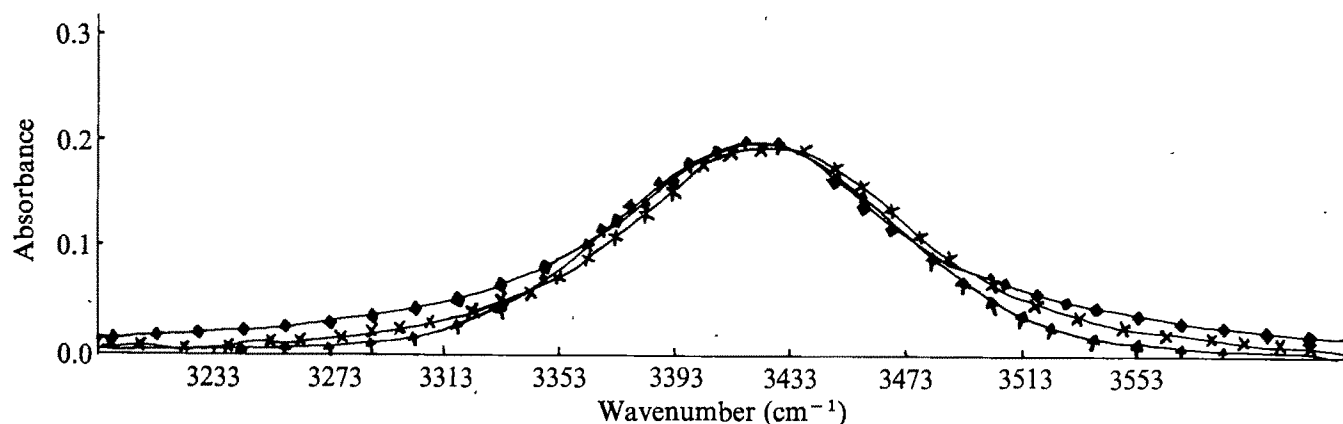


Fig. 1 Comparison of observed ν_s band profile (x) with those of pure Gaussian (\uparrow) and pure Lorentzian (\blacklozenge) bands for phenol-acetonitrile in carbon tetrachloride

random force $F(t)$. A complete derivation of equation (2), indicating the limits of its applicability, is being prepared for publication elsewhere.

For small t this correlation function behaves as

$$|\varphi(t)| = \exp(-\frac{1}{2}\Delta^2 t^2) \quad (3)$$

and for large t it has the asymptotic form

$$|\varphi(t)| \sim C \exp(-\Delta^2 \tau_c t) \quad (4)$$

where the amplitude of modulation Δ and the correlation time τ_c of the $\nu_s(\text{XH}\cdots\text{Y})$ vibration are given by

$$\Delta = a\langle r_2^{-2} \rangle^{1/2} \quad (5)$$

and φ

$$\tau_c = \beta/\omega_2^2 \quad (6)$$

According to Kubo's general theory of frequency modulation by a Gaussian random process⁶, the former expression for $|\varphi(t)|$ is a good approximation for all times if $\tau_c \Delta \gg 1$, the latter if $\tau_c \Delta \ll 1$. In these limiting cases of slow and rapid modulation the spectral profiles are Gaussian and Lorentzian respectively. Published theoretical work on the band shapes of hydrogen-bonded species in solution has either assumed the extreme slow modulation limit⁴ or has attempted to interpolate between the two limiting cases⁷.

The experimental autocorrelation function for the phenol-acetonitrile complex (Fig. 2) shows clearly that neither limiting case is strictly applicable. Since the $\nu_s(\text{XH})$ band in monomeric phenol is close to Lorentzian in form, it seems likely that quite different mechanisms are responsible for the band shapes of the monomer and the complex. This provides indirect support for the view that the coupling of internal modes of the complex influences its spectrum, which is the basic assumption of the present model. Local field effects may well be of some importance⁸, but are not considered here.

Fitting the experimental autocorrelation function to expression (2) above, using the nonlinear least-squares method of Marquardt⁹ as modified by Fletcher and Powell^{10,11}, we obtain the following estimates of the parameters for phenol-acetonitrile in solution in carbon tetrachloride at 22.5 °C:

$$\Delta = (14.8 \pm 0.2) \times 10^{12} \text{ s}^{-1}$$

$$\Delta^2 \tau_c = (11.9 \pm 0.4) \times 10^{12} \text{ s}^{-1}$$

and

$$\bar{\nu}_2 = 120 \pm 12 \text{ cm}^{-1}$$

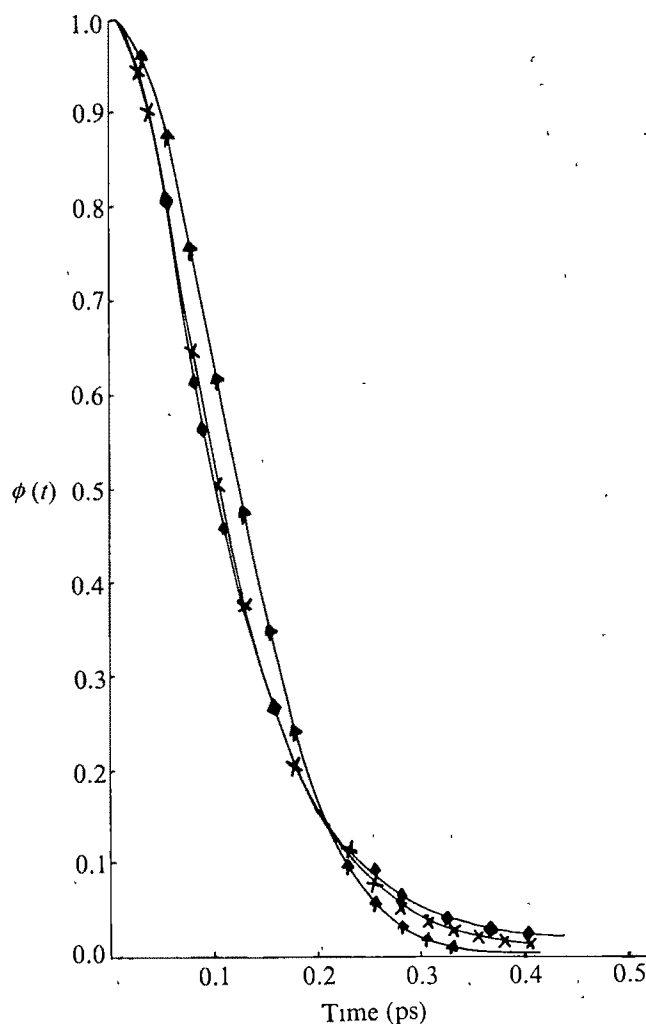
$$\text{where } \bar{\nu}_2 = \omega_2/2\pi c$$

It follows that $\tau_c \Delta = 0.8$ and $\beta/2\omega_2 = 0.6$. We observe that $\tau_c \Delta$ is close to unity, which confirms that neither the slow nor the rapid modulation limiting approximation is satisfactory.

Although the $\nu_s(\text{XH}\cdots\text{Y})$ motion is underdamped, it is not far from being critically damped, and this suggests that the far infrared spectrum of the complex in solution should be extremely broad. We have failed so far to observe the far infrared spectrum.

It should be particularly noted that we are able to calculate the standard deviation of our measurement of the hydrogen bond stretching frequency. Although we cannot exclude the

Fig. 2 Comparison of observed dipole correlation functions obtained by transformation of the spectral bands shown in Fig. 1.



possibility of systematic errors (arising from the crudity of the theoretical result on which the analysis is based, or even from bias in our sampling procedure) we must point out that no other method of measuring the $\nu_s(\text{XH}\cdots\text{Y})$ frequency allows a reliable estimate of the errors of observation to be made. We believe therefore that our procedure must be regarded—by default—as the most trustworthy method yet devised for measuring the hydrogen bond stretching frequency of a complex in solution. It must be admitted, however, that the model makes no provision for any spectral broadening produced by local field effects or by the involvement of rotational and bending degrees of freedom, and that these questions need urgent attention.

We have not attempted in this preliminary report to discuss the temperature dependence of the position of the $\nu_s(\text{XH})$ band centre, although it is relatively straightforward to do so by introducing a further coupling term in the model hamiltonian.

There are several ways in which the model may be tested more stringently. First, the modulation amplitude parameter Δ of equation (5) should decrease by a factor of nearly $2^{-1/2}$ on deuteration. This we have verified for the phenol-dioxan system. Secondly, changing the solvent or varying the amount of excess base (which we have found to produce distinct spectral changes, thus confirming previous work on similar systems^{12,13}) should affect the viscous damping parameter β and thus affect the correlation time τ_c of equation (6).

Finally, changes in temperature should affect both Δ and β . The former, being proportional to the root mean square amplitude of a quantum mechanical harmonic oscillator, should vary as $[\coth(\hbar\omega_s/2kT)]^{1/2}$, or as $T^{1/2}$ at sufficiently high temperatures. The latter, being a viscosity coefficient, is expected to decrease exponentially with increasing temperature. Further experimental work on these problems is in progress and will be reported at a later date, together with a more detailed analysis of experimental errors.

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Effect of noise on shock-elicited aggression in rats

ONLY a limited number of experiments have been designed to evaluate the effects of noise on aggression. As animal models of aggression are amenable to pharmacological and physiological analysis we have investigated the effect of noise on aggression in rats and have found an interesting non-monotonic relationship, with an increase in aggression at moderate noise levels but a decrease at high levels. The aggressive behaviour chosen for

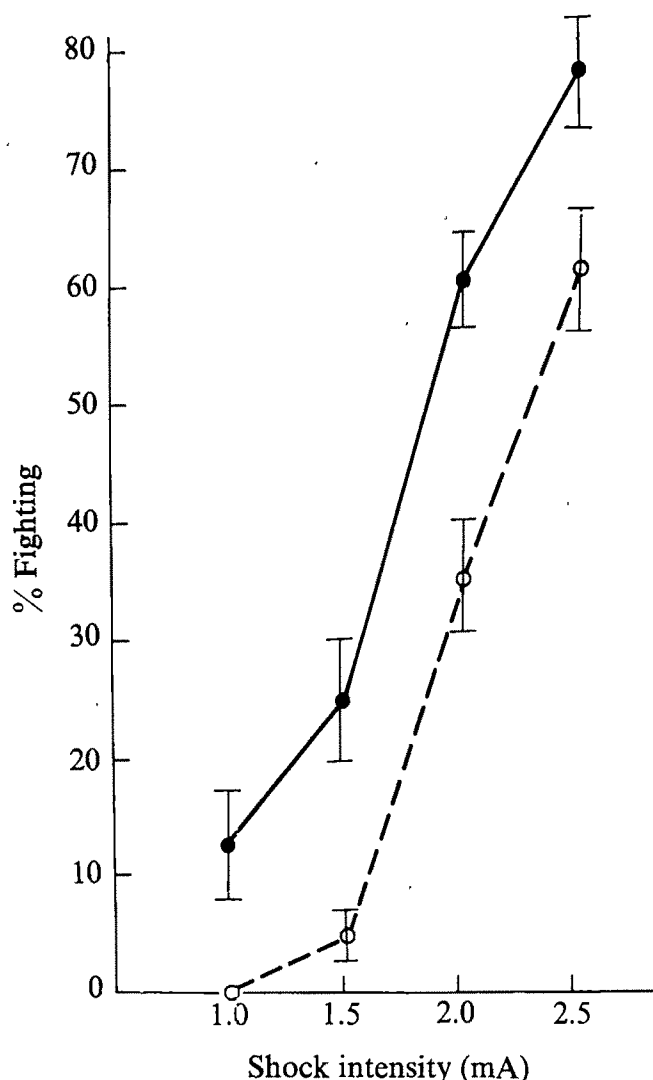


Fig. 1 Mean percentage fighting \pm s.e.m. at each of four shock intensities, 10 shocks each at 1, 1.5, 2 and 2.5 mA when the background noise was either 75 dB (solid) or 55 dB (dotted) for eight pairs of rats. Duration of shock 1 s, 15 s between shocks, 2 min between each shock intensity. Sprague-Dawley male rats 300–350 g, $30 \times 28 \times 24$ cm Plexiglas test cage in sound-attenuated chamber. White noise intensity measured by General Radio Model 1551-C sound level meter. The pairs of rats placed in test chamber were not previously housed together. Half the pairs received 75 dB first, half 55 dB. Three days later pairs retested in reverse conditions.

the present experiments was shock-elicited aggression in the rat. Two rats were paired in a small enclosure and subjected to a series of footshocks which elicit fighting, depending on the intensity, frequency and duration of electric shock^{1,2}. This is a well documented and highly reliable form of aggressive behaviour that is usually considered a form of irritable aggression³.

The results of Experiment 1 are shown in Fig. 1, which plots mean percentage fighting $[(\text{observed fights}/\text{total possible fights}) \times 100]$ at each of four shock intensities when the background noise level was either 75 or 55 dB. The number of fights increased as the intensity of shock increased but at each shock intensity the number of fights was greater in the 75 dB compared with the 55 dB condition ($F(\text{shock intensity}) = 60.9$; d.f. = 3/21; $P < 0.001$; $F(\text{noise}) = 23.46$; d.f. = 1/7; $P < 0.001$; $F(\text{interaction}) = 1.22$; d.f. = 3/21; $P > 0.10$). This experiment indicates therefore that footshock-elicited aggression was increased by raising the level of background noise.

It is possible that noise increases shock-elicited aggression by increasing arousal. Many arousal mechanisms exhibit non-monotonic relationships to stimulus intensity where the behavioural effect is greatest at intermediate levels of stimulus

intensity, but less at low or high levels of stimulus intensity. For example, the relationship between the startle response and background noise is a non-monotonic one^{4,5}. Both the startle response and shock-elicited aggression may be considered to reflect types of irritability which might be influenced by shifts in arousal. As background noise may be an effective means of manipulating arousal, it was of interest to determine whether shock-elicited aggression would also bear a non-monotonic relationship to the level of background noise. Experiment 2 was designed to evaluate this possibility.

Figure 2 shows the mean percent of fights across the 30 shocks at each of three levels of background noise and indicates that the relationship between this measure of aggression and noise level was a non-monotonic one ($F(\text{quad}) = 110.37$; d.f. = 1/16; $P < 0.001$). Thus, there was an increase in aggression when the noise level was raised from 55 to 75 dB ($t = 3.40$; d.f. = 8; $P < 0.001$), but a decrease in aggression when noise level was raised to 90 dB ($t = 5.21$; d.f. = 8; $P < 0.001$). In addition, there was even less fighting at the high intensity of background noise than at the low intensity ($t = 2.11$; d.f. = 8; $P < 0.05$).

Experiment 3 was designed to evaluate whether the length of exposure to noise before testing would influence subsequent aggression. Different groups ($n = 9$) were exposed to either 55 or 75 dB noise for either 1, 5 or 15 min. After pre-exposure, a series of 30, 2 mA shocks were presented at the same noise level used during pre-exposure. The results indicated that although there were highly significant increases in the number of fights during 75 compared with 55 dB noise conditions ($P < 0.001$ in each group) there was no significant effect of varying the length of pre-exposure to noise before testing.

Experiment 4 was designed to evaluate how long the increase in aggression that occurred when testing was carried out at the 75 dB noise level would continue, once that noise was turned down. Nine pairs of rats were each given a series of 40, 2-mA shocks at a 15-s intershock interval 5 min after being placed in the test cage. For three pairs the background noise was 75 dB throughout, for three other pairs it was 55 dB throughout. For

the last three pairs the noise was 75 dB for the first 5 min but 55 dB thereafter. The various pairs were then tested in the other two conditions 3 and 6 d later using a 3×3 latin square to determine the order of testing.

The following results were obtained. When rats were exposed to 75 dB throughout the test period the amount of fighting increased to a maximum level in 2.5 min and remained at this level thereafter. Rats exposed to the 75 dB noise level which was then reduced to 55 dB also quickly increased their frequency of fighting to a peak at 2.5 min. Thereafter the frequency of fighting rapidly declined, so that after approximately 3.75 min it was equal to that of rats exposed to 55 dB throughout. The last two experiments indicate therefore that the effect of noise on shock-elicited aggression is most dependent on the level of noise present during testing where the effect develops and decays very rapidly.

The present series of experiments demonstrate that noise can influence shock-elicited aggressive behaviour. The precise mechanism which mediates this effect remains to be investigated. One possibility might be that noise increases arousal. Increasing arousal by electrical stimulation of the reticular formation⁶ or by amphetamine⁷ has been shown to facilitate aggressive behaviour in the cat. Moreover, the relationship between arousal and behaviour often seems to follow an inverted U function⁸ where increases in arousal at first increase the frequency and/or intensity of behaviour but further increases in arousal actually decrease the same behaviour.

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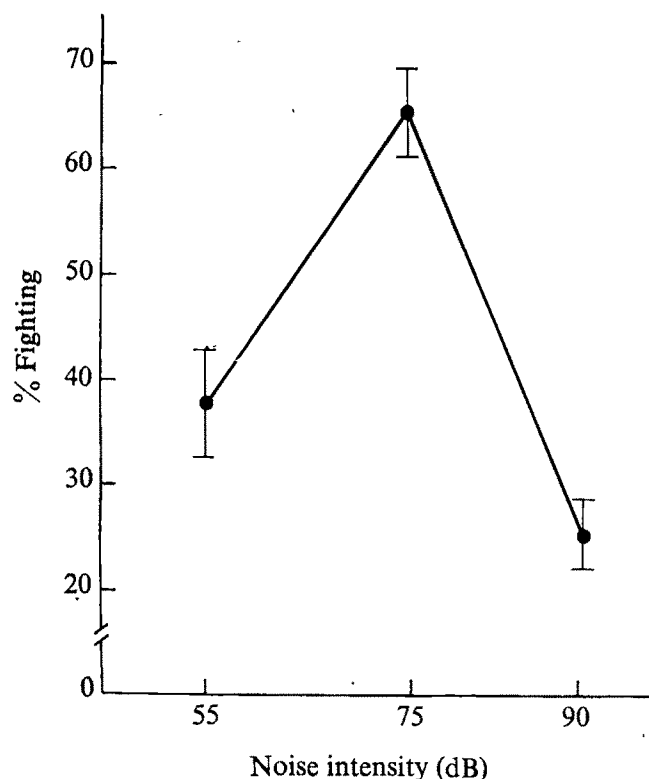
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Fig. 2 Mean percentage fighting \pm s.e.m. across 30 shocks at 55, 75 and 90 dB background noise. Nine pairs of rats. Design: 3×3 latin square, testing at each noise level followed every other level equally often. 30, 2 mA shocks given at 15 s intervals. The 55 dB point represents baseline noise level with the noise generator turned completely off.



Strain differences in maze-learning ability of *Drosophila melanogaster*

If it could be demonstrated that *Drosophila* are able to learn, then our extensive knowledge of the genetics of this dipteran could be applied to understanding the biological bases of learning. Reports of learning in *Drosophila*¹⁻³ sometimes involve changes in orientation as the result of aversive stimulation, for example, electric shock. The use of these stimuli can bring additional problems such as conditioned inhibition where the animals cease responding altogether⁴. The present paper is the first report of work on a unique learning situation which is based on the well-known mazes used to study taxes in *Drosophila*⁵ and involves no aversive stimulation.

We have used modifications of 11-unit mazes (Fig. 1), where flies are allowed 24 h to travel from the starting tube to the collection tubes, making 10 right-left choices at junctions connected by one-way cones. Gravitational and extraneous light cues were eliminated by mounting each maze horizontally, with an opaque base, sides and top. The internal parts of the maze were of clear Perspex which allowed the flies to be attracted through the maze by phototaxis, the light source being a 10 W fluorescent lamp, the width of the maze, shining

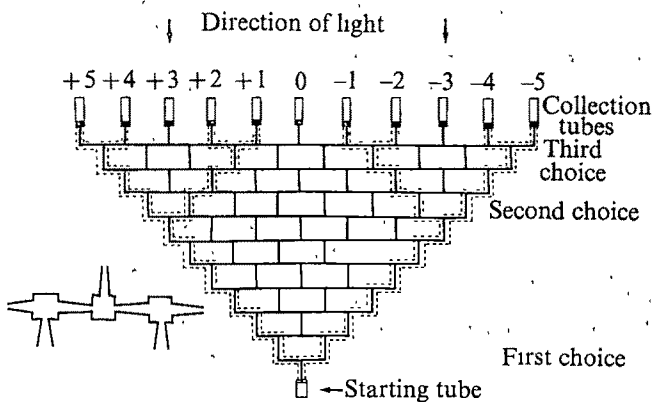


Fig. 1 Schematic drawing of the maze. Dashed lines represent the possible pathways in the modified maze, where the other alternatives are blocked off. Dimensions: 62×49 cm. Small drawing shows a typical junction with the interconnecting one-way cones.

from behind the collection tubes. In such a maze, one does not know the sequence of turns made by the flies. If the number of choices is reduced from 10 to 3, however, by blocking off certain junctions of the maze (Fig. 1), the flies are limited to eight alternative pathways, corresponding to the eight collection tubes, tubes +3, 0 and -3 no longer being used.

This modified maze was used to study the sequence of choices in five replicates of each of 10 strains of *D. melanogaster*, LM20-29, derived from single inseminated females captured 9 yr previously at Leslie Manor in Victoria Australia and inbred mainly by single-pair matings since that time. Genetic variation for other traits, such as mating speed, chaetae number and desiccation resistance exists between these strains⁶. Because locomotor activity changes in young flies⁷, all testing was carried out 3 d after eclosion: 100 males and 100 females of the same strain were put in the starting tube (diameter 2 cm, depth 1 cm), providing sufficient crowding to induce the flies to enter the maze. Compared with other experiments where only 50 (ref. 2) or 60% (ref. 8) of the flies completed the task in the allotted time, an average $92.1 \pm 7.8\%$ of these 200 flies reached the collection tubes (10 × 2.5 cm culture vials) at the other end of the maze.

Unlike conventional maze studies where subjects are run through the same maze several times, the flies here were only

tested once. The index of learning involved comparing the probabilities of going left or right at the start of the maze and the probabilities of repeating this response at the second choice-point indicated in Fig. 1, termed PL, PR, PLL and PRR, respectively. Between these two choices the flies were forced to make six turns, to the left in the case of those initially turning left and to the right for those initially going right. If the behaviour of the flies is unaffected by the six forced turns, then the values of PLL and PRR should be similar to those of PL and PR, any change being denoted by $CL = PLL - PL$ and $CR = PRR - PR$. Significant values of CL and CR indicate that when presented with a choice, flies are more likely to continue the sequence of turns they were forced to adopt for the previous six turns. That is, they learn that this sequence of turns enables them to proceed through the maze towards the light, which presumably also acts as an orientational cue in their turning.

All the necessary statistics can be calculated from the distribution of flies in the eight collection tubes. PL is the proportion out of all the flies in these eight tubes that are in the four tubes on the left side and that therefore must initially have turned left. PLL is calculated as the proportion out of the flies from the four left-side tubes that are in the two furthest left tubes (+5 and +4). PR and PRR are calculated similarly for those flies initially turning right.

Analyses of variance (Table 1) revealed significant differences between the strains in PL, the initial tendency to go left. Moreover, as found in earlier work⁶, flies are initially more likely to turn left than right (overall mean PL = 0.5446). Such a turning bias has been observed in other arthropods⁹ and cannot simply be attributed here to asymmetry of the maze, as any such influences would be the same for each strain (the testing of the five trials of each strain were randomised between three identical mazes, each completely separated by matt-white partitions from the other mazes)—that is, asymmetry alone does not explain the observed differences in PL among the strains.

From the significant strain differences in CL and CR (Table 1), we see that after six forced turns to the left or right, the flies of some strains are more likely to choose to continue turning this way. The overall mean values for CL and CR of 0.1644 and 0.2655 respectively indicate that the increase in this probability is considerably less for left- than for right-turning, although the latter was initially the less frequent choice.

Flies that learn follow the outer walls of the maze, and it is necessary to consider alternative factors to learning that could lead to flies behaving in this way. One crucial experiment demonstrates that such alternatives could not contribute to the strain differences in learning.

Table 1 Values of PL, CL and CR for the ten inbred strains, and analyses of variance

Strain	PL	CL	CR
LM20	0.5592±0.0458	0.2888±0.0696†	0.3729±0.0776†
LM21	0.5737±0.0703	0.1970±0.1153	0.3231±0.0900*
LM22	0.5491±0.0606	0.0945±0.0874	0.1701±0.1033
LM23	0.5191±0.0452	0.1586±0.0943	0.2631±0.0801*
LM24	0.5015±0.0545	0.3273±0.0781†	0.3009±0.0948*
LM25	0.5666±0.0646	0.1139±0.1403	0.3139±0.0768†
LM26	0.6053±0.0493	0.0318±0.0707	0.3254±0.1066*
LM27	0.4500±0.0574	0.2261±0.0588*	0.1511±0.0814
LM28	0.5407±0.0654	0.1435±0.1382	0.1458±0.0794
LM29	0.5810±0.0467	0.0623±0.0748	0.2881±0.0969*
Item	d.f.	Mean square	Mean square
Strain	9	3.61*	9.20†
Sex	1	0.061	3.89†
Strain			
× sex	9	0.92	0.39
Between replicates	80	1.60	0.65

PL is the initial probability of turning left, CL the change in this probability after an initial left choice and six forced turns and CR the corresponding change in the probability of going right after an initial right turn. Means s.e. of five runs of 200 flies (100 of each sex). The values of CL and CR which differ significantly from zero are indicated thus (d.f. = 4 in all cases), * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

Mean squares for analyses of variance have been multiplied by 100. The between-replicates variance could be used as an error variance only for PL, as the corresponding variances for CL and CR differed significantly between the strains, those with low means having larger variances, presumably because their choices were more random. Transformations of the data were attempted but did not reduce heterogeneity of the variances.

Five sets of 200 flies (100 of each sex) of strains LM20, 26, 27 and 28—which vary from both CL and CR being significant to neither being significant (Table 1)—were tested in the normal 11-unit maze with no passages blocked off and where flies thus were not forced to repeat a sequence of turns. Otherwise the mazes were identical. The mean percentages \pm s.e. of flies ending up in one or another of the outermost tubes (that is, +5, +4, -4, -5) were, for each of the above strains, 35.6 ± 3.9 , 42.8 ± 4.7 , 21.4 ± 1.9 and 30.8 ± 1.5 , respectively. Although these results do show that some strains are more likely than others to follow the outside wall and so end up in the end tubes, these strain differences bear no relationship to the differences in CL and CR in the eight-unit maze—that is, large values of CL and CR in strains like LM20 cannot simply be the result of flies following the maze walls without learning.

That other strains like LM28 follow the walls to the same extent as LM20 in this maze but show no significant CL or CR in the eight-unit maze eliminates explanations of the learning by factors such as a light gradient, where flies merely follow the outer walls because of cues provided by light getting through the black Perspex baffles at each side of the maze or shining from the maze end, but being reflected differentially from the outer and inner maze surfaces. Although they are already excluded by this 11-unit maze experiment, there is some more specific evidence to eliminate other possible alternatives to learning for the eight-unit maze performance:

Stampede effect, where flies follow each other in groups³. At 2 and 4 h after starting the 200 flies in each maze, the means \pm s.d. of the number of flies having reached the collection tubes were 49.5 ± 34.1 and 89.8 ± 28.7 , respectively. On both occasions the distribution of flies matched the final distribution after 24 h. This would not have been the case if following were of major importance as the distribution should then show clumps of flies in particular collection tubes.

Wall-hugging (following the outer walls of the maze) and its converse, **centrifugal swing** affect maze performance in other invertebrates¹⁰. These factors may be relevant in other *Drosophila* maze studies^{8,11} but are less likely here, where the junctions have many small corners with both curved and flat surfaces to be negotiated (Fig. 1, detail). Moreover, observations of flies in transparent-topped mazes show that they can explore a junction for as long as 45 min before proceeding, rather than going straight through the maze, as a wall-hugging and centrifugal swing hypothesis would require.

Right-left bias. At the initial choice point, flies are being separated by their preference for turning left or right and it is simply those strains with a greater initial bias in one direction or another that are more likely to repeat the choice at the second choice-point. Turning seems to be probabilistic, however, and there is no evidence that some individual flies consistently turn left and others right. All the flies reaching the +5 tube during the five trials of each strain were stored on fresh food and rerun through the maze (-5 flies were similarly treated). If the bias explanation were correct, then the +5 and -5 flies would be those with a strong bias to turning left or right respectively at choice-points in the maze. Presumably this bias should be indicated on the rerun by increases in the initial probability of going left (PL) for the +5 flies, and right (PR) for the -5 flies, but in none of the 10 strains were the values of PL and PR significantly different from the original values in Table 1. Similarly, 27 generations of separate selection (from an F_2 population) of flies going to +5 or -5 has produced some increase in CL and CR, but not the change in PL and PR that a bias explanation would predict (D.A.H., unpublished). Also, if a right-left bias were important, the probabilities of repeating the same response at the third and final choice-point should be higher than the earlier probabilities. The opposite is the case, although possibly this third choice is affected by the flies being close to the end of the maze and being able to smell the food medium in the collection tubes (see Fig. 1) and so need not imply that learning has been lost by this stage of the maze.

Sequential alternation. When an organism is forced to turn one way in a maze and then given a choice, it will sometimes turn the opposite way, demonstrating what is termed sequential alternation⁴ or correcting behaviour¹². Although this may be considered a form of learning—the animal must retain some memory of the way it was forced to turn—and could be important in other mazes where passages are not blocked off and where *Drosophila* do repeat turns or sequences of turns⁸⁻¹¹, it cannot be the sole explanation here, for the same reasons that wall-hugging and centrifugal swing have been excluded.

Additional possibilities such as the olfactory cues postulated in other maze studies¹³ have been tested and excluded. The remaining explanation is that the flies display associative learning. With no apparent positive or negative reinforcement, they associate passage through the maze towards the light with a sequence of turns or, more accurately, with a particular orientation towards the light at each choice-point, since, as mentioned previously, flies may move about the junction for a considerable time before actually making the choice. Thus the behaviour here can be considered a form of the exploratory learning which is well-documented in the feeding and homing

of other arthropods¹⁴. Putnam⁹ has explained similar behaviour in the beetle *Aleochara bilineata* as evidence of learning—the beetles make the first choice in a Y maze at random, but the probability of repeating this choice increases on successive trials even without extrinsic reinforcement. He ruled out alternatives like right-left bias and odour cues discussed here, and concluded the beetles learn they can proceed through the maze by turning in this direction. As they never try turning the opposite way at a choice point, they do not find that this alternative response would be equally effective. Both there and in the present mazes, mere progress seems to constitute some differential reinforcement for repeating the same choice. Putnam also refers to work on rodent learning in mazes with reinforcement in both arms that can be explained in the same way.

Thus, genetic differences in learning have been demonstrated in *Drosophila*. The learning task is unusual but allows large numbers of flies to be scored, as it only requires the introduction of flies into the start tube and scoring 24 h later of the final distribution in the collection tubes. As there is no other interference by the experimenter, it should prove easier to replicate these results than has been the case with other *Drosophila* learning situations¹⁵. Like the aversive conditioning techniques¹⁻³ learning is being considered here at the population, rather than the individual level. But there has already been considerable success in the genetic analysis of taxes in mazes similar to these⁵, and the present technique may offer an effective screen for localising chromosomal factors determining the learning ability of *Drosophila*.

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Two photosystems controlling behavioural responses of *Halobacterium halobium*

Halobacterium halobium contains a retinal-protein complex, bacteriorhodopsin, which is the only protein of the so-called purple membrane forming distinct patches within the surface membrane^{1,2}. Bacteriorhodopsin acts as a photoreceptor molecule and is chemically similar to the visual pigment rhodopsin^{1,3}. On illumination bacteriorhodopsin undergoes a fast cyclic photoreaction with at least four intermediates occurring after microseconds and milliseconds⁴⁻⁶. Various experiments indicate that bacteriorhodopsin functions as a light-driven proton pump which builds up a proton gradient across the cell membrane and is used for ATP synthesis^{7,8}. Thus its primary function, different from that of rhodopsin in the eye, seems to be energy transformation⁷. Moreover, *H. halobium* shows light dependent motor responses, and so we supposed that bacteriorhodopsin also has a sensory function. To test this possibility we obtained action spectra for the light-induced behavioural responses from *H. halobium*. We

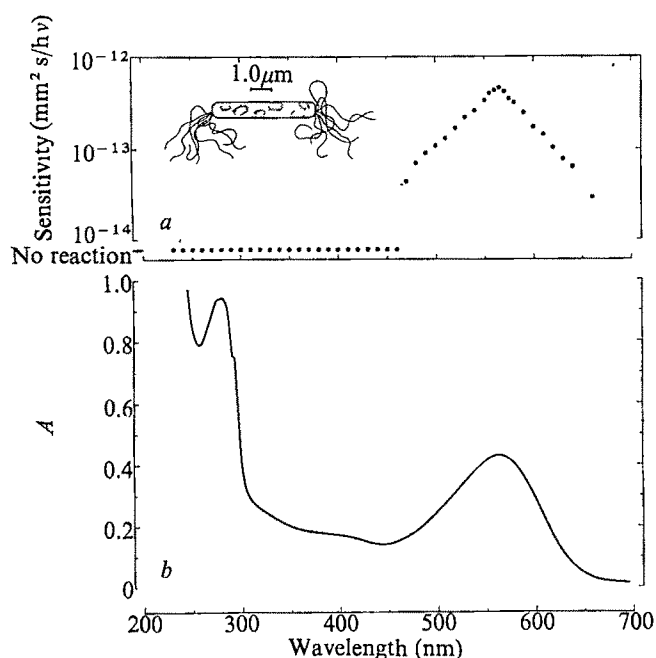


Fig. 1 *a*, Action spectrum of the inverse photophobic response (IPR) of *H. halobium* occurring after decrease of light intensity. Background illumination ($\lambda = 420\text{--}490\text{ nm}$) was given through the condenser of a phase contrast microscope. Stimulating light (half width $\leq 10\text{ nm}$) generated by a xenon source (XBO 150 W) or a mercury lamp (HBO 150 W) in connection with a high intensity grating monochromator, Bausch and Lomb, visible range, or a Zeiss monochromator type M4 QIII, respectively, was applied through a quartz objective connected with an incident light illuminator. Light intensity was measured by means of a light detector UDT 40 connected with pin 10 or pin 10 ultraviolet calibrated against a thermopile. Ordinate: sensitivity in log units given as the reciprocal of the change in light intensity which evokes the response after 1.9–2.0 s. Standard deviation of the means does not exceed the diameter of the points ($n = 4$). Room temperature. *b*, Absorption spectrum of the isolated purple membrane (bacteriorhodopsin) in H_2O after purification on a linear sucrose density gradient.

found two photosystems, one of which shows that bacteriorhodopsin is involved in the light-controlled motor response.

Experiments were performed with the mutant strain R_1 of *H. halobium*, lacking gas vacuoles. The bacteria were usually in the stationary growth phase, during which a maximum of purple membrane is present. These rod-shaped bacteria are about 4–10 μm long and 0.5–0.7 μm in diameter. Tufts of flagella on both poles, visible in electron micrographs of negatively stained bacteria, enable the cell to swim in both directions of its long axis. The mean swimming rate was 2.3 $\mu\text{m s}^{-1}$ (at 24 °C). Sudden changes in light intensity elicit a phobic response. After a latent period of about 1 s the bacterium stops moving for about 1 s and after axial rotation starts swimming in roughly the opposite direction. Similar responses can be evoked either by an increase of light intensity, called⁹ the direct photophobic response (DPR), or by a decrease of light intensity, the inverse photophobic response (IPR). In both cases the time elapsed between the beginning of stimulus and the first visible response depends on intensity, duration and wavelength of the stimulus.

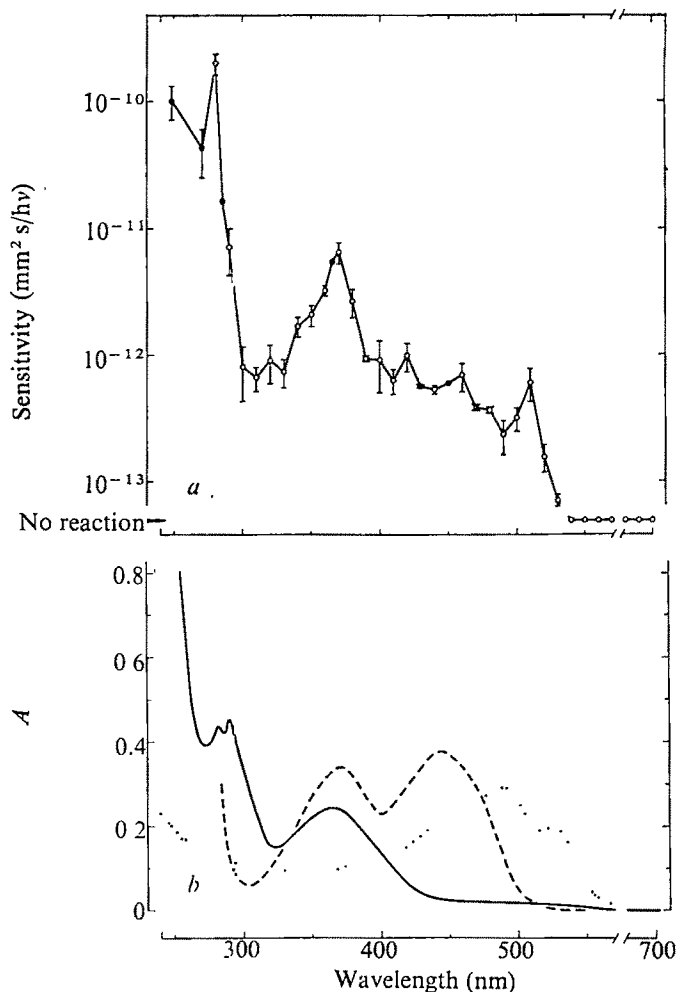
Action spectra of the DPR and the IPR were obtained by measuring the stimulus intensity at the threshold. Single bacteria in a suspension were observed microscopically and the stimulus intensity at different wavelengths was varied until 19 out of 20 specimens examined showed the stop reaction within the time interval from 1.9 to 2.0 s after the onset of stimulus. Stimuli were always maintained until completion of the phobic response.

The action spectrum of the IPR (Fig. 1*a*) shows a single maximum of sensitivity at 565 nm which corresponds to the peak of absorbance of bacteriorhodopsin in the visible (Fig. 1*b*).

At wavelengths shorter than 460 nm no IPR could be elicited. We call this photosystem PS 565 and assume that its effective pigment is bacteriorhodopsin. This interpretation is supported by the fact that bacteria from young cultures which have not yet synthesised the purple membrane did not show the IPR. The absence of a 280-nm band in the action spectrum can be explained by (1) the probably insufficient intensity in the ultraviolet of the light source and (2) the limited energy transfer from the protein part to the excitable site of the pigment molecule, as found in frog rhodopsin¹⁰.

In the conditions described above a decrease of light intensity of 720 nW mm^{-2} , that is 2×10^{12} quanta $\text{mm}^{-2} \text{s}^{-1}$, at 565 nm was required to elicit the IPR. From the dependence of the threshold on the latent period the absolute threshold intensity was calculated to be 2×10^{11} quanta $\text{mm}^{-2} \text{s}^{-1}$, corresponding to an estimated 6×10^5 quanta per bacterium per s or 2 quanta per molecule bacteriorhodopsin per s. This relatively low sensitivity compared with that of animal photoreceptor cells indicates that amplification is absent or much less developed. Neither intensity nor wavelength of background illumination affected the height of the threshold. Thus adaptation defined

Fig. 2 *a*, Action spectrum of the direct photophobic response (DPR) of *H. halobium* occurring after increase of light intensity. Background illumination $\lambda > 645\text{ nm}$; half width of stimulating light 0.5–5.0 nm. Ordinate: Sensitivity as in Fig. 1*a*. Mean values of 3–11 measurements (\circ) or single values and means of two (\bullet). Vertical bars represent the standard deviation of the mean. Room temperature. *b*, Absorption spectra of a retinylidene protein obtained after treatment of the purple membrane with 10 mM CTAB (cetyltrimethyl-ammoniumbromide) at $\text{pH} \geq 8.5$ (—), of riboflavin in H_2O (---) and of methanol/ether extraction of pigments from *H. halobium* (mainly α -bacterioruberine) after separation of the purple membrane (....)



as change in sensitivity due to background illumination is absent in PS 565.

The action spectrum of the DPR (Fig. 2a) has two distinct maxima at 280 and 370 nm and smaller secondary peaks towards longer wavelengths. Beyond 530 nm no response could be evoked. With regard to the major peaks we believe a chromoprotein to be the effective pigment of this photosystem, which we call PS 370. The action spectrum resembles the absorption spectrum of a retinylidene protein which can be obtained by CTAB treatment of the purple membrane at alkaline pH (ref. 1) (Fig. 2b). The small peaks in the action spectrum beyond 400 nm may be due to the large amount of carotenoids, mainly α -bacterioruberine¹¹ (Fig. 2b) which could participate in the photoreception or act as a shielding pigment. If the latter is true, the photoreceptor pigment could be a flavoprotein (Fig. 2b), which we consider the more likely because of the marked decrease in sensitivity close to 500 nm. A flavoprotein has also been proposed as a photopigment for other bacteria¹².

To elicit the DPR within a latent period of 2 s a stimulus intensity of 80 ± 10 (s.d.) nW mm⁻², that is $(1.5 \pm 0.2) \times 10^{11}$ quanta mm⁻² s⁻¹ ($n = 7$) at 370 nm and of 3.5 ± 0.8 nW mm⁻², that is $(5.0 \pm 1.2) \times 10^9$ quanta mm⁻² s⁻¹ ($n = 11$) at 280 nm was required. The absolute threshold at 370 nm was calculated to be 1.5×10^{10} quanta mm⁻² s⁻¹. With blue background illumination ($\lambda = 420$ –490 nm) the threshold was 50 times that with red background illumination ($\lambda > 645$ nm) of equal intensity (4 mW mm⁻²). PS 370 developed about 10 h earlier than PS 565 and reached maximal sensitivity immediately whereas the latter was maximally sensitive after 40–50 h. When stimulus intensity and duration were varied the product of both (Bunsen–Roscoe rule) at threshold was constant in PS 370, but not in PS 565.

Our experiments demonstrate that *H. halobium* has two photosystems, as also suggested by H. Berg (personal communication). Both trigger flagellar responses and can be considered as sensory mechanisms. A sensory function implies that the stimulus controls a process which is provided with energy from other sources, that is that the important process is not transduction of energy but of information. Signal amplification, however, as in photoreceptor cells of animals seems not to be essential. Thus, besides the energy-converting function, bacteriorhodopsin acts as a signal transducer.

At present we have no clear idea how sensory transduction may function in *H. halobium*. In PS 565 the excitation could be triggered directly either by light-induced changes in the proton gradient or by an electrical potential across the cell membrane, which might be generated by the electrogenic proton pumping function of bacteriorhodopsin^{13–15}. Control of the motor response through a decrease of stored cellular energy, however, seems unlikely because, among other reasons, of the relatively short latent period even with green background illumination of high intensity, which should keep the cellular energy at a constantly high level. Nevertheless, the response could be triggered by one step of the energy-converting mechanism. If the photopigment of PS 370 is a flavin, a change in electron transport must be taken into account, as has been discussed for the light-induced tumbling effect in *Salmonella typhimurium*¹². By analogy with other cells exhibiting sensory properties, such as receptor cells and protozoa, it should be remembered that changes in the permeability of the cell membrane for certain ions could be mediated by the photosystems and that resulting passive ion fluxes lead to changes in flagellar activity.

The biological relevance of the sensory function is obvious for PS 565. The bacteria do not respond when entering an area of visible light but always reverse their direction if they cross to the dark. Thus they will be trapped in the light which can be used for ATP synthesis. By means of PS 370 the bacteria could avoid ultraviolet radiation which would damage the organisms, as has been shown with mutants lacking carotenoids¹⁶.

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Correlation of electrical and mechanical responses in nervous control of cilia

ALTHOUGH cilia have many important functions in the majority of animal phyla, our knowledge about the mechanism by which their activity is controlled has been fragmentary¹. Extensive electrophysiological studies of the ciliated cell, with intracellular microelectrodes, have been made only with the relatively large protozoans, principally *Paramecium*, where the ciliary activity has been shown to be closely associated with the electrical properties of the cell membrane^{2,3}. Among the metazoans, some examples of nervous control of ciliary activity have been reported⁴, but, mainly because of technical difficulties, intracellular recording of the membrane potential from the ciliated cells has been made in only a few cases^{5,6}; in no case has nervous control of cilia been studied by recording simultaneously the electrical and the mechanical events. Such recording is essential to understanding the mechanism of nervous control at the cellular level.

Using the nerve–cilia preparation made from the gill of the bivalve mollusc, *Mytilus edulis*, we have been able to obtain simultaneous records of neurally evoked mechanical and electrical responses of the ciliated cell. The 'lateral' cilia in this preparation have been known to show an abrupt inhibition of motion, the ciliary arrest response⁷, in response to nerve stimulation, as well as an increased beat frequency which usually follows prolonged repetitive stimulation of the nerve⁸.

A nerve–cilia preparation, consisting of the visceral ganglion and a few gill filaments linked together by a branchial nerve, was dissected out as described previously⁷. From this a nerve-supplied single gill filament preparation was obtained by removing all gill filaments except one. The gill filament was then held, with one of its lateral surfaces facing downward, on the underside of a coverslip placed over a specially designed chamber. A glass capillary electrode with a vertically bent tip, filled with 1.5 M potassium citrate and having a resistance higher than 140 M Ω , was used to penetrate the lateral cell from below. Electrical stimuli were given to the nerve at a distance of 17–20 mm from the site of microelectrode penetration. The mechanical activity of cilia was monitored by projecting their microscopic image through a pinhole on to a photomultiplier tube. The output from the photomultiplier and the membrane potential of the lateral cell were simultaneously recorded on magnetic tape or photographed directly from the oscilloscope screen. The distance between the sites

of recording of the electrical and the mechanical events was 10–20 μm .

With successful penetration, a more or less steady resting membrane potential of about 60 mV (inside negative) was obtained; penetrations which gave potentials less than 30 mV were discarded as these were often followed by a slow depolarisation to zero level. There was no change in the membrane potential corresponding to the cyclic beating of cilia.

When the nerve was stimulated with a 2-ms pulse, there was a transient depolarisation of the lateral cell membrane which took place after a latency of about 120 ms, accompanied by a quick arrest response of the lateral cilia as shown in Fig. 1. The electrical response could be recorded only from the ciliated lateral cells and not from any adjacent regions of the epithelium. The electrical response lasted some 200 ms, of which the depolarising phase (time to peak) was about 30 ms. The amplitude of the response was not constant, but was usually less than 20 mV (mean of 26 measurements, 12.5 mV, 22–25 °C). No overshoot was recorded throughout the experiments. There was a marked facilitation of the electrical response when the nerve was stimulated at about 5 Hz, and also a summation evident at 20–40 Hz.

Repetitive stimulation elicited a prolonged arrest response of the lateral cilia, even when the individual electrical responses were separated (Fig. 2). The mechanical response usually outlasted the repeated electrical responses. On the other hand, the onset of the electrical response preceded that of the mechanical response by about 30 ms. The mechanical response was observed when the electrical response was above about 2 mV, although the threshold depolarisation, as well as the latency, of the mechanical response could not be determined accurately with the present monitoring method.

These results indicate that the transient depolarisation of the lateral cell induced by nervous activity is correlated with the quick arrest response of the cilia, and not with the frequency increase nor with the initiation of each beat. We have previously shown that the response can be induced by a high concentration of potassium⁹, and also by an 'outward' electric current passed across the ciliated membrane¹⁰. The present results are in full accord with these and support the view that the animal can control the cilia by depolarising the cell membrane⁹. They also support the work of Mackie *et al.*⁶ who recorded electrical activity associated with the ciliary arrest response in the ascidian *Corella*.

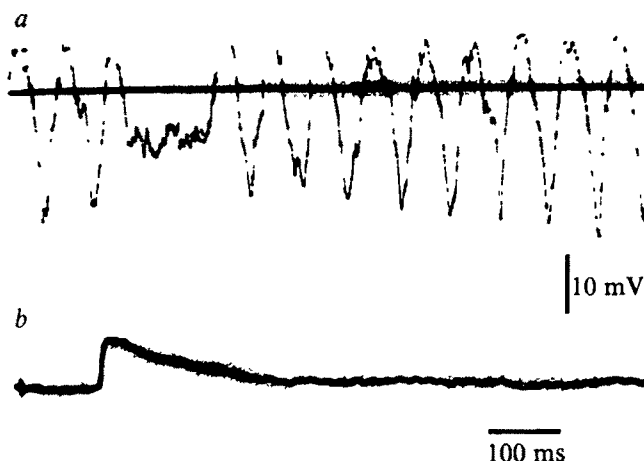


Fig. 1 Ciliary arrest response in the gill of *Mytilus*, elicited by stimulating the branchial nerve with 2-ms pulse given at the beginning of the record *a*. Photoelectrically recorded activity of lateral cilia. Prevailing oscillatory pattern represents normal beating activity, momentarily suppressed after stimulation *b*. Intracellular recording of the membrane potential. Note that the onset of electrical response (depolarisation) precedes the mechanical response. Resting membrane potential: –60 mV (21 °C). A reference line indicating –10 mV is superimposed on *a*.

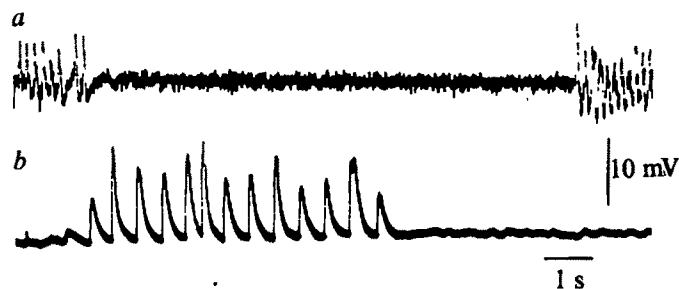


Fig. 2 Response to repetitive stimulation of the branchial nerve at about 2 Hz. *a*, Ciliary activity; *b*, membrane potential. Note that the ciliary arrest response outlasts the electrical activity. Resting membrane potential: –67 mV (26 °C).

In *Paramecium*, calcium has been known to play an essential role in the coupling between membrane depolarisation and the 'reversal' response of cilia³. In *Mytilus*, too, the arrest response seems to depend on calcium since it disappears in calcium-free media¹¹ and also since, in the 'model' cilia made by treatment with Triton X-100, a similar response can be induced by adding calcium ions to the medium¹². The mechanism underlying the arrest response seems to be fundamentally similar to that underlying the ciliary reversal response in Protozoa, as has already been pointed out^{6,9,13}. The ciliary reversal response in certain metazoans is also correlated with the electrical activity of the membrane^{14,15}.

To conclude, the present results support the hypothesis that the ciliary arrest response, as well as the reversal response, is controlled by a mechanism comparable with that which regulates the muscular contraction through membrane excitation and the action of calcium ions^{3,13}.

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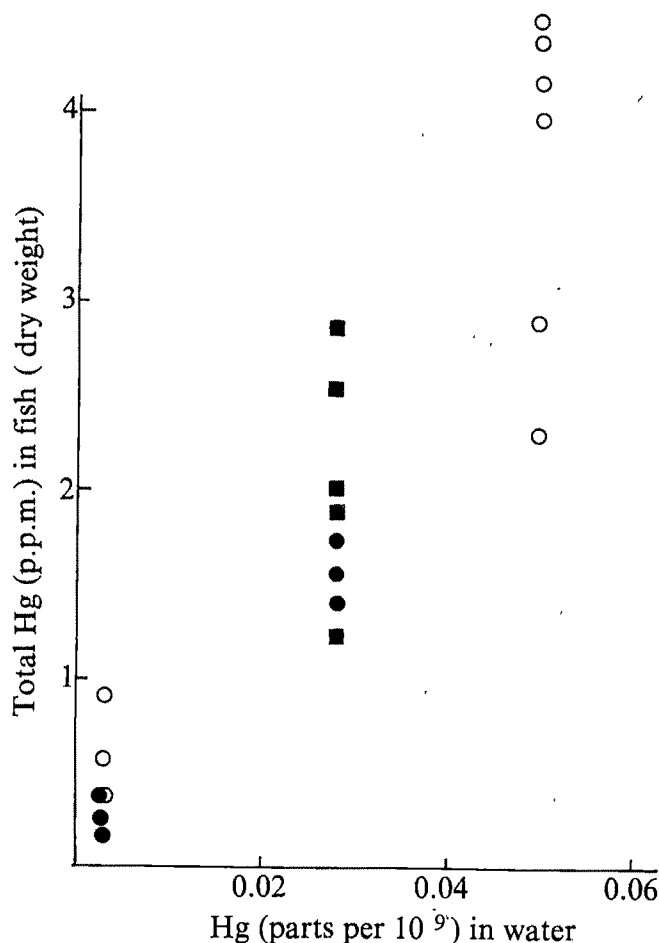
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Concentration of mercury by three species of fish from Japanese rivers

THE concentration of mercury in aquatic organisms is quite high relative to that in the surroundings but the exact mechanism by which this effect occurs is not known. Generally, higher mercury levels in the environment are reflected in the amount found in protoplasm. Mercury levels in rivers and lakes have increased markedly as a result of industrial discharges; thus, there is a proportionality between mercury concentrations in fishes and in waters, it is possible, using permissible mercury concentrations in fish tissue as a guide (Japan 0.4, US 0.5 p.p.m.), to

make recommendations for acceptable mercury levels in lakes and rivers. I have found no reports regarding this relationship.

Mercury contents of two rivers receiving waste discharged from mercury mines of an unpolluted river, and of three species of fish (crucian carp, *Carassius carassius langsdorffii*; dace, *Triblodonhakonensis*; zacco temmincku, (Temmincket Schlegel)) feeding mainly on organic matter in water, were determined. Rivers studied were the Hoono in Nara Prefecture, and the



increases in SO_2 concentrations in the presence of oxidants may result in a potential health hazard.

Subjects were eight 19–25-yr-old normal, non-smoking male university students with no history of significant chest illness or atopic illness. They were exposed to SO_2 and O_3 in a chamber that has been described before². Ozone was generated by electrical discharge in oxygen, and SO_2 (partially diluted with air) was added to the chamber from a cylinder. SO_2 and O_3 were added to the inlet of the chamber, and both gases were sampled about 2 feet from the nose of the subjects. Since O_3 will interfere with the analysis of SO_2 and *vice versa* we used scrubbers connected to the intake tubing of the analysers to remove the interfering gas.

SO_2 was removed from the intake, sample tube to the O_3 meter (Mast model 724-2) by a scrubber of glass fibre paper impregnated with chromium trioxide in 60–70% sulphuric acid. O_3 was removed from the sampling tube to the SO_2 meter (SI model 67) by a scrubber of crystals of ferrous sulphate heptahydrate.

As the exposure chamber was in a laboratory, in an air-conditioned building without externally opening windows, we assumed that the temperature and humidity varied little from day to day, and there was negligible particulate pollution. Since the atmospheric levels of SO_2 and O_3 were below 0.1 p.p.m. at the time of exposures, the contamination of the experimental atmosphere was unlikely.

All experiments followed the same protocol. Control pulmonary function was tested at time zero, and subjects then exercised on a bicycle ergometer at a work rate sufficient to double their ventilation for 15-min periods, alternating with 15-min rest periods for 2 h. They were tested again at 30-min intervals from the onset of exposure (0.5, 1.0, 1.5, 2.0 h) with a final test 30 min after the end of exposure.

Forced expiratory flow–volume curves were recorded by a Fleisch No. 3 pneumotachograph connected to a differential pressure transducer (HP model 270) and coupled to a carrier preamplifier (HP model 8805A). The resulting flow signal was displayed against volume on a storage oscilloscope equipped with dual trace amplifiers, volume being obtained by integration of flow signal.

This volume signal was recorded simultaneously on a four-channel FM tape recorder for later tracing of a spiogram. Four flow–volume curves were recorded on each occasion, and the definitive value was taken as the mean of the two largest values of the four. In addition to the dynamic lung function tests, six slow vital capacity curves were measured, three before and three after the exposure.

We have already described the effect of 0.37 p.p.m. of ozone

on human pulmonary function, finding a just significant decrease in maximal mid-expiratory flow rate (MMFR) at the end of 2 h (ref. 3). We now report that SO_2 , when present alone at a concentration of 0.37 p.p.m. had no significant effect on ventilatory function in 2 h. These results are shown in Fig. 1, which also shows the decline in this measurement of ventilatory function when both gases were present together. Clearly, at concentrations of this magnitude, the two gases together have a much greater effect than does either individually.

As in previous tests of human response to air pollutants, there was considerable individual variation. The largest decrease in maximal mid-expiratory flow rate was from an initial value of 7.93 l s^{-1} to 2.78 l s^{-1} at the end of 2 h, and the smallest reduction was from a control value of 8.74 l s^{-1} to 7.98 l s^{-1} after 2 h. For the group as a whole, the change after 30 min of exposure (from a mean of 6.49 with s.d. of ± 1.72 to a mean of 5.60 with s.d. of ± 1.63) was already significant at the 2% level. Other measurements of ventilatory function computed from either expiratory flow–volume curves or spiograms also decreased progressively during the same 2-h experiments.

The forced expiratory volume in 1 s ($\text{FEV}_{1.0}$) decreased to 78% of its initial value at the end of 2 h when both gases were present; the mid-expiratory flow rate, at 50% of vital capacity, decreased to 54% of its initial value in this atmosphere and the peak expiratory flow rate decreased to 79% of its initial value. The vital capacity, measured as a static, non-forced parameter of function, was 92% of its initial value at the end of 2 h exposure for the group as a whole. The decrease in the flow measurements was thus proportionately much greater than the decrease in static volume.

The enhancement of the toxicity of SO_2 and O_3 , present together, has been known for plants for some years⁴. As the simplest approximation, one can imagine the two gases interacting rapidly in the humidified air of the airways and on the large, wet air–tissue interface of the lung, painting the inner surface with sulphuric acid.

Are these experiments reliable indicators of what may be happening outside the laboratory? Lebowitz *et al.*⁵ have demonstrated a decline in ventilatory function in children and adolescents after outdoor exercise on days of relatively high pollution in Tucson, Arizona. Their air pollution data suggest that the interaction of oxidants and sulphur dioxide and sulphate produce the effect, since the levels of nitrogen dioxide and particles were not high enough to cause decline in function.

Are these experiments useful indicators of acceptable and unacceptable environments? At the very least, we can conclude that it is unwise to consider quantitatively acceptable levels for single pollutants when synergism among various pollutants may occur. Nevertheless, in our view, those environments with high enough levels of mixed pollutants to cause discomfort or even adverse changes in pulmonary function on moderate physical activity, should not be acceptable. Furthermore, we can safely assume that the long-term consequences of such repetitive insults may well result in serious lung damage.

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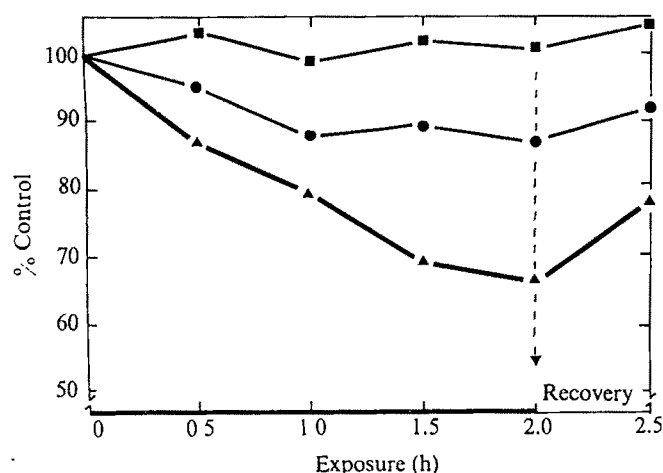
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Fig. 1 Effect of SO_2 and O_3 , separately and together, on maximal mid-expiratory flow rate in intermittently exercising non-smokers. The arrow indicates the beginning of the recovery period. ■, 0.37 p.p.m. SO_2 ($n = 4$); ●, 0.37 p.p.m. O_3 ($n = 8$); ▲, 0.37 p.p.m. $\text{SO}_2 + \text{O}_3$ ($n = 8$).



Immunologically-mediated restraint of latent tumour metastases

DIFFERENT grafted sarcomas, all originally from chemically-induced autochthonous tumours, vary widely in their capacity to form metastases, as determined by the incidence of secondaries which appear in the lung and lymph nodes in the months following surgical removal of the 'primary' transplant from syngeneic rats¹. Tumours which do not form metastases readily in normal animals can be induced to do so by grafting into syngeneic rats depleted of T lymphocytes by procedures which do not compromise the bone marrow¹. We concluded that specific immune processes requiring the participation of T lymphocytes contributed to the control of metastatic spread—possibly by reducing the number of tumour cells released because there is an inverse relationship between the number of macrophages within the tumour and metastasis^{1,2}. In addition, circulating tumour cells may be destroyed immunologically and an immunologically-specific factor that acted against intravenously-injected sarcoma cells was demonstrated in the serum and lymph of tumour-bearing rats^{3,4}.

The experiments which we now report illustrate a further mechanism by which host immunity may control the development of distant metastases. Pure-line Hooded rats received transplants of the syngeneic 3,4-benz-pyrene-induced sarcoma HSBPA into the left leg, and 2 weeks later the tumours and their local draining lymph nodes were removed by amputation of the whole limb as described previously¹. Figure 1 shows that approximately 10% of the animals so treated died of distant metastases 55–100 d after removal of the tumour, the rest remaining tumour-free during the observation period of 18 months (that is, more than half of their normal lifespan). If the rats were exposed to either whole-body X irradiation, or to 7 d continuous draining of the thoracic duct lymph at different times after the removal of the primary transplant (the longest interval so far studied being 1 month), however, there was a highly significant increase in the incidence of distant metastases compared with the control group. In addition, it was noted that individual animals had more tumour lesions in both lymph nodes and lungs than control animals, and that their deaths due

to metastases frequently occurred earlier—especially in groups treated soon after surgery.

Although both whole-body irradiation and severe lymph depletion affect many different aspects of the animal's physiology, their main common feature is that of suppression of lymphocyte-mediated immunity, and it seems likely that this factor is responsible for their similar effects on the growth of metastases. Reconstitution experiments to determine whether the induction of metastases by these procedures can be reversed by giving syngeneic lymphocytes are in progress.

The fact that immunosuppressive treatments, even when given 1 month after surgical removal of a tumour, increase the incidence of metastases, excludes the possibility that in this case we are dealing with an effect on circulating tumour cells. We conclude that even sarcomas which normally are essentially non-metastatic shed cells which lodge and survive in the lung and lymph nodes, where they remain in a viable state without growing into macroscopically detectable lesions. Immunosuppression of the host permits such cells to develop into lethal tumours. This observation does not preclude some form of immunological control before these cells lodge themselves in the lung and lymph nodes, as the incidence of metastases reported here for postsurgically-immunosuppressed animals is not as high as that reported previously for the same tumour when immune suppression was applied during the period of tumour growth¹.

The existence of dormant cells which retain the potential to grow in rats from which the primary transplants have been surgically removed is paradoxical, as these same rats are capable of rejecting inocula of up to 10^7 cells given intramuscularly¹. Indeed, the claim that the tumour is highly immunogenic rests on this finding. We are now attempting to discover if these latent metastases persist as single cells, avascular clusters, or microfoci which are undergoing cyclical growth and regression. One fact seems clear—that at least half of the animals, seemingly cured surgically, carry dormant tumours that manifest only after treatments which are immunosuppressive. These experiments suggest a cautious approach to the use of chemotherapy for dealing with disseminated cancer cells which may be present after all clinically detectable tumour has been removed by surgery or radiotherapy, as such prophylactic systemic chemotherapy is often immunosuppressive. Prophylactic chemo-

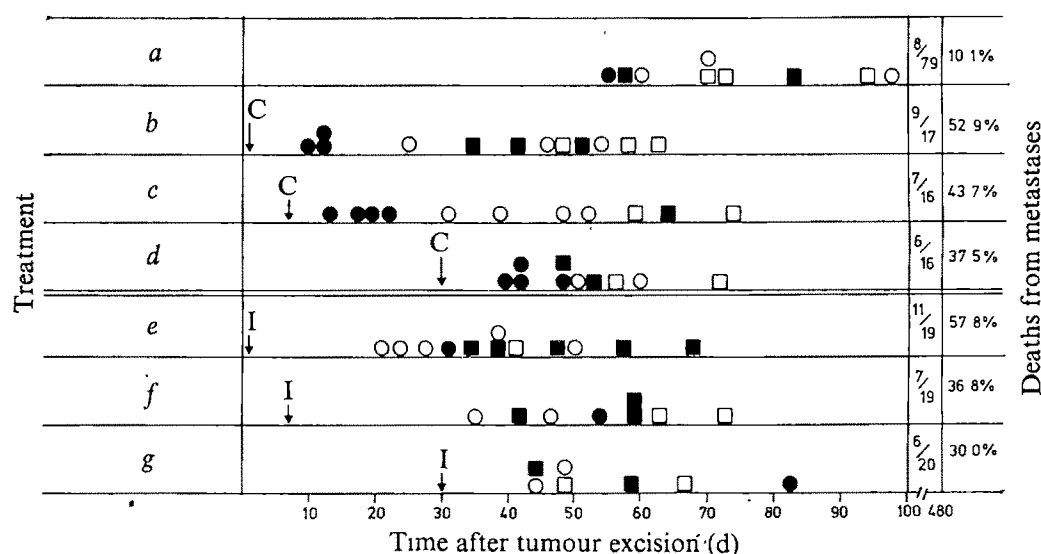


Fig. 1 Effect of postsurgical immunosuppression on incidence of metastases after excision of HSBPA tumour. HSBPA tumours were grown intramuscularly in hind limbs of Hooded rats for 14 d, and removed by amputation of the whole limb. Immunosuppressive procedures were initiated 1, 7 or 30 d after tumour excision. These were: chronic thoracic duct lymph drainage (lymph was drained for 7 d during which time $1-3 \times 10^8$ lymphocytes were removed, and the cannulae were then tied off); and whole-body irradiation (rats were given

one sublethal dose of 500 rad X irradiation from a conventional Marconi machine at 220 kV, 15 mA and 54 cm FSD). *a*, No treatment (controls; $n = 80$); *b-d*, thoracic duct lymph drainage ($n = 20$ in each case); *b*, days 1–7; *c*, days 7–14; *d*, days 30–37; *e-g*, whole-body irradiation: *e*, day 1; *f*, day 7; *g*, day 30. Deaths: ●, with no evidence of metastases; ○, with lymph node metastases; ■, with lymph node and lung metastases; □, with lung metastases. I, irradiation carried out on days indicated by arrows

therapy is clearly of value when progressive microlesions are present, which if not eradicated would definitely develop into overt metastases; but it may be harmful when the disseminated tumour cells are dormant.

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Stimulation of hepatocytes and lymphocytes *in vitro* by liver regeneration

We have reported the presence of serum factor triggering DNA synthesis in partially hepatectomised rats, using a parabiosis with direct aortic cross circulation¹. The finding was subsequently confirmed with experiments both *in vivo*² and *in vitro*³. We have also reported that partial hepatectomy stimulated function of lymphoid tissues and increased the number of antibody-forming cells in the spleen of rats immunised with sheep red blood cells⁴. It was then not certain how hepatectomy modifies the immune reaction, but now we present evidence that the humoral factor stimulates both hepatocytes and lymphocytes in culture.

Male Lewis rats (80-100 g) were subjected to 70% partial hepatectomy under ether anaesthesia in the morning⁵. Sham operation consisted of an abdominal incision and gentle palpation of the liver. All rats were anaesthetised again with ether 8 h after operation and were exsanguinated by cardiac puncture. Pooled blood was stored overnight at 4 °C, and then the serum was obtained by centrifugation. The serum was kept frozen at -20 °C until used.

Primary liver cell cultures were derived from livers of Lewis rat foetuses at 18-20 d gestation. Single cell suspensions were prepared after collagenase digestion of the tissue⁶. The viability of the cells was 85%, as measured by the Trypan blue exclusion test. The cells were grown in Falcon plastic dishes (35 mm) containing Eagle's MEM which was supplemented with non-essential amino acids, antibiotics, and 10% heat-inactivated foetal calf serum (Grand Island Biological). The initial cell inoculum was 5×10^5 per 2 ml per dish and maintained at 37 °C in a 10% CO₂-air incubator. The medium was changed 24 and 48 h after plating. At 4 d, the old medium was removed from the cultures and the dishes were washed with serum-free medium. Fresh medium (2 ml) containing the serum to be tested at a final concentration of 10%, was added to the culture along with ³H-thymidine (2.5 µCi ml⁻¹). After incubation at 37 °C for 24 h, the medium was removed and the dishes washed twice with Tris buffered saline (pH 7.4). A sample (2 ml) of a 0.05% trypsin solution in Ca²⁺- and Mg-free Tris buffer

were added to the dish and incubated at 37 °C for 30 min. The resulting cell suspension was used to determine the cell number and radioactivity of acid-precipitable material in a Packard scintillation counter. Identical experiments were performed with the foetal kidney cell culture.

To test the effect of partially hepatectomised serum on lymphocyte replication, lymphocyte suspensions were made from the cervical and mesenteric lymph nodes of Lewis rats. The culture medium used was the same for the foetal liver cell culture except that 10% heat inactivated rat serum (pooled from BN strain) was used instead of the foetal calf serum. To show that the 10% foetal calf serum was mitogenic to the rat lymphocyte in our hands, it was substituted for the rat serum which gave a low control value. The initial suspension contained 1×10^6 cells per 2 ml in sterile Falcon plastic culture tubes. The cells were cultured undisturbed for 4 d and supplemented with fresh medium containing the serum in question as mentioned above. At the end of the culture (day 5) smears of cell suspensions were made and stained with Wright-Giemsa. The percentage of mitoses was determined in counts of 1,000 cells. DNA synthesis was also estimated in all experiments by the incorporation of ³H-thymidine.

Results of the ³H-thymidine incorporation into liver cells and lymphocytes are shown in Table 1. A marked increase of DNA synthesis was seen in the liver cells cultured with partially hepatectomised (PH) serum (Table 1, left column). Serum taken from either sham-operated syngeneic (Lewis) or allogeneic (BN) rats was also shown to be mitogenic to Lewis foetal liver cells, compared with the cells cultured without the rat serum. The results strongly favour the hypothesis that partial hepatectomy increased mitogenic serum factor³ but does not decrease inhibitor. No mitogenic effect of the PH serum was observed on the kidney cell culture (data not shown).

Surprisingly the PH serum was also stimulatory to lymphocytes (Table 1, right column). This effect is highly significant compared with either normal rat serum ($P < 0.001$) or sham-operated rat serum ($P < 0.001$). All three sera (NR, PH, and sham) showed significantly high uptake when compared with the culture without the serum. The stimulatory effect of the hepatectomised serum on lymphocyte replication was first seen after the cells were cultured for 8 h or more.

The number of cells in mitosis is shown in Table 2. The serum from partially hepatectomised rats stimulated mitosis of both liver cells and lymphocytes in culture significantly more ($P < 0.001$) than the sera from either normal or sham-operated rats.

Evidence suggesting the presence of regeneration factor in the serum of partially hepatectomised rats was first provided by Bucher *et al.*⁶ in experiments with simple parabiotic rats connected only with skin and muscles. It was firmly confirmed by a cross circulation of aortae of parabiotic rats; a significant increase in DNA synthesis in the normal liver of a parabiotic partner after a 70% hepatectomy of the other^{1,2}. Stimulating effect of the regeneration serum on the growth of fibroblasts⁷, cultured rat hepatocytes⁸ and foetal liver cells³ has since been reported.

Table 1 Stimulation of DNA synthesis as measured by incorporation of ³H-thymidine into hepatocytes and lymphocytes *in vitro* in the presence of serum from partially hepatectomised rats

Experiment	Foetal liver cells (c.p.m. per 0.5×10^6 cells per 24 h)				Lymphocytes (c.p.m. per 10^6 cells per 24 h)			
	NR serum	PH serum	Sham serum	No serum	NR serum	PH serum	Sham serum	No serum
1	6,350	13,100	5,190	2,380	2,000	5,130	1,750	1,060
2	9,100	17,500	6,500	3,590	2,150	4,200	2,010	870
3	5,300	11,000	5,900	4,070	1,550	3,950	1,310	690
4	6,100	9,500	4,850	3,410	1,900	4,100	1,550	930
5	5,050	12,600	3,760	1,820	1,610	3,500	1,600	770

Liver cells (left column) were cultured in 35-mm plastic dishes in 2 ml of Eagle's medium supplemented with 10% heat-inactivated foetal calf serum. On day 4, the old medium was pipetted and the cultures incubated for 24 h with 2 ml of medium containing either 10% normal rat (NR), partially hepatectomised (PH), sham-operated rat sera or no serum along with ³H-thymidine (2.5 µCi ml⁻¹). Lymph node lymphocytes (right column) were cultured in 5-ml plastic tubes in 2 ml of the medium with 10% heat-inactivated pooled homologous serum. Addition of the serum to be tested and assay for DNA synthesis were the same. Each experiment was done on 3 rats. Average of 3 dishes is shown for each experiment.

Table 2 The mitotic rate of hepatocytes and lymphocytes cultured with partially hepatectomised rat serum for 24 h.

Serum source	Mitosis (% mean \pm s.d.)	
	Foetal liver cells	Lymphocytes
Normal rats	4.83 \pm 2.01	0.28 \pm 0.16
Partially hepatectomised rats	9.05 \pm 2.64	0.87 \pm 0.20
Sham-operated rats	4.29 \pm 1.87	0.31 \pm 0.15
None	3.56 \pm 1.08	0.12 \pm 0.08

Culture conditions for both liver cells and lymphocytes are the same as shown in Table 1. At the end of the culture (on the day 5) smears of the cell suspensions were made and stained with Wright-Giemsa. The percentage of the mitosis was determined per 1,000 cells. The data represent means with standard deviations from total 15 cultures for each serum tested.

Partial hepatectomy leads to increased DNA synthesis not only in the regenerating liver cells but also in lymphoid tissues as measured by ^3H -thymidine uptake⁹. We have shown that partial hepatectomy affects the recruitment of thymus cells on the one hand, but on the other, it increases the number of antibody-forming cells by shortening their doubling time^{4,10}. It was then suspected that the regeneration serum might be mitogenic to both the hepatocyte and lymphocyte. For a technical reason in the present experiment, both types of cells, foetal liver cells and adult lymph node cells were treated slightly differently in that the former were cultured after collagenase digestion of the tissue and in a 10% foetal calf serum medium, whereas the latter were cultured in a 10% rat serum medium without the enzymatic digestion. While it might be argued that the enzyme and different serum sources might have triggered the membrane receptors of both cell types differently, these culture conditions would not be a decisive factor, since only the PH serum could stimulate the DNA synthesis of both cells when added at the end of the fourth day and cultured more than 8 h.

The breakdown of the peripheral lymphocytes took place 7-8 h after partial hepatectomy and the peak of RNA synthesis in the liver appeared at the same time. It is therefore possible that the breakdown product of the lymphocyte liberates other active agents independent of hepatotrophic factor, which stimulate increased precursor cell proliferation of the lymphocyte. Although the fact that the PH serum did not stimulate the cultured kidney cells suggests a hepatolymphatic interrelationship, it remains to be seen whether the serum factor(s) appearing after partial hepatectomy is multipotential, one factor triggering the hepatocyte replication and the other stimulating the lymphocyte, or unipotent, a factor regulating both cell types.

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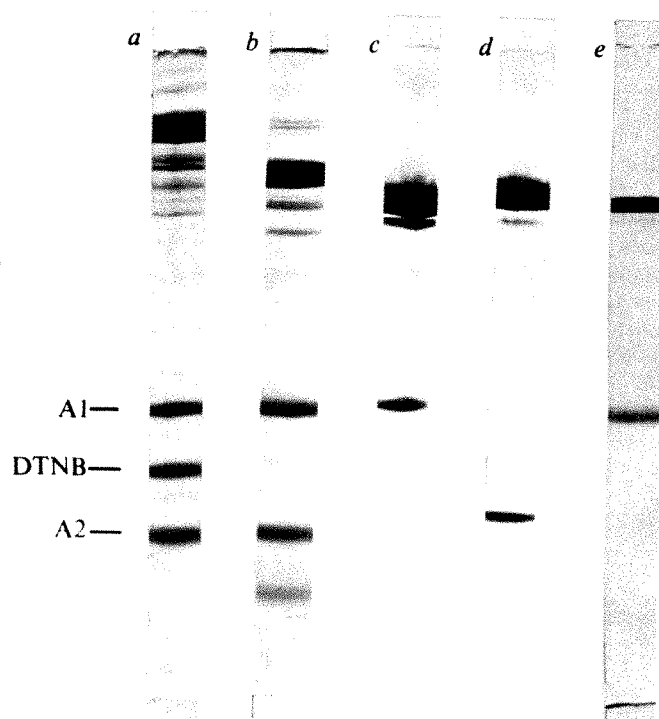
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Separation of subfragment-1 isoenzymes from rabbit skeletal muscle myosin

RABBIT skeletal muscle myosin is composed of two heavy chains and four light chains¹. Two classes of light chains can be distinguished both chemically and functionally. Within the class of essential light chains (those which cannot be removed without loss of ATPase activity), two chemically related but phenotypically distinct proteins have been identified², both of which are present in single fibres of fast-twitch muscles³. The existence of both phenotypes within a single psoas muscle cell indicates that either there is a single population of myosin molecules with a different alkali light chain on each subfragment-1 head, or there are at least two populations of myosin present. Densitometric and radiochemical methods have shown that there is an unequal distribution of these two light chains; this supports the hypothesis that myosin isoenzymes occur in histochemically homogeneous muscles³⁻⁵ which cannot be attributed to the presence of contaminating slow-twitch fibres. The presence of different heavy chains also is indicated by the observation of amino acid substitutions in certain peptide sequences^{6,7}. Thus fast-twitch muscles contain myosin showing heterogeneity of both light and heavy chains, suggesting isoenzyme populations, but so far these have not been separated, nor have the proteolytic subfragments derived from them.

The smallest active fragment obtainable from myosin is the globular head called subfragment-1 (S-1), which is usually produced by brief papain digestion⁸. This subfragment has been used extensively for kinetic analysis of the ATPase activity⁹. Unfortunately it is even more heterogeneous than its parent myosin. Partial loss of the non-essential DTNB light chain is commonly observed on exposure of myosin to papain⁸, and the

Fig. 1 Polyacrylamide gel electrophoresis on 10% gels run in 0.1 M Tris-bicene and 0.1% SDS and stained as described previously³. a, Chymotryptic HMM; b, chymotryptic S-1; c, fraction S-1(A1) pooled from the DEAE-cellulose column; d, fraction S-1(A2) from the column; e, S-1(A1) from a single peak tube showing one heavy chain and the alkali 1 light chain.



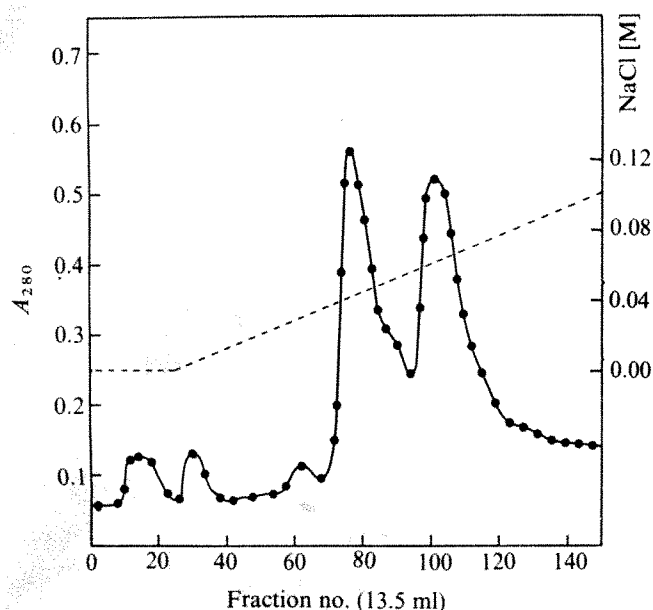


Fig. 2 Chromatography on DEAE-cellulose (Whatman DE-52) was carried out at 4 °C using a 48 × 2.5-cm column in 50 mM imidazole-HCl buffer at pH 7.0. This S-1 was eluted with a linear gradient to 0.12 M NaCl and the absorbance was measured at 280 nm.

heavy chains show the presence of nicks when subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS)^{8,10}.

To characterise the different isoenzymes of myosin it is essential to separate these S-1 fragments, and further to obtain species which do not contain adventitious proteolytic cleavage of the heavy chains. This has not proved possible using papain, but following some observations of Yagi and Otani¹¹, we have developed methods to produce selective cleavage of the myosin head, and have also been able to eliminate the DTNB light chains from the resultant S-1. Two populations of S-1 molecules are obtained; one contains the alkali 1 light chain [S-1(A1)] and the other, alkali 2 [S-1(A2)], and both give single heavy chain bands on polyacrylamide gels. The two populations have been separated by chromatography, modifying the procedure described previously⁸.

Rabbit myosin was prepared as described⁸, and digestion for 10 min at 25 °C in 0.6 M NaCl, 10 mM sodium phosphate, pH 7.0, at 10–20 mg ml⁻¹ using α-chymotrypsin at 0.05 mg ml⁻¹ produced heavy meromyosin (HMM), which was isolated in the supernatant after dialysis to an ionic strength less than 0.05. (Digestion was terminated by addition of 100 mM phenyl methane sulphonyl fluoride in ethyl alcohol to a concentration of 10⁻⁴ M.) Gel electrophoresis of this HMM is shown in Fig. 1; it contained a single heavy chain and three light chain bands. If the digestion was carried out on myosin filaments produced by dialysis to 0.12 M NaCl, 20 mM sodium phosphate, pH 7.0, and containing 1 mM EDTA, HMM was no longer produced; instead the product was S-1, which on gels showed a single heavy chain band and only two light chains (Fig. 1). Thus under these conditions the susceptibility of myosin to chymotrypsin is altered, and the S-1 obtained is both more homogeneous and less degraded than that produced by papain digestion.

With the DTNB light chain absent, we have been able to fractionate the two components in this S-1 preparation (Fig. 2). Gel electrophoresis of the products is shown in Fig. 1. Although the second fraction frequently contains some contaminating S-1(A1), the first fraction is remarkably clean, yielding a single heavy chain and one light chain which comigrates with alkali 1 light chain marker. The molecular weights obtained by low speed sedimentation equilibrium for these two components were in the range 116,000–126,000, similar to values obtained for papain S-1 (ref. 8).

ATPase activity was measured to investigate kinetic differences between the two S-1 fractions. The calcium ATPase, measured with a pH-stat, in 30 mM KCl, 10 mM CaCl₂ and 1 mM ATP at pH 8.0, showed no significant differences. For example, in one preparation the turnover number of S-1(A1) was 8.1 s⁻¹ and that of S-1(A2), 8.2 s⁻¹ (both based on a molecular weight of 115,000). In an earlier preparation which was not fractionated, the activity of 7.7 s⁻¹ was identical to that obtained for a parallel preparation of papain S-1.

The magnesium-dependent ATPase activity, measured by a linked assay system¹², again showed no significant differences between these fractions. The steady state rate was always in the range 0.04–0.05 s⁻¹, similar to published values for papain S-1 (ref. 12). When ATPase measurements were made in the presence of actin, striking differences were observed. In three different preparations we found that the V_{max} for S-1(A1) was about half that obtained for S-1(A2). One such experiment is shown in Fig. 3. The regression line extrapolates to give $V_{max} = 28$ s⁻¹ for S-1(A1), and 54 s⁻¹ for S-1(A2), while K_{app} , determined from the intercept on the abscissa is 10 μM actin for S-1(A1) and 50 μM actin for S-1(A2). By comparison, the V_{max} obtained for papain S-1 under these conditions was 40 s⁻¹.

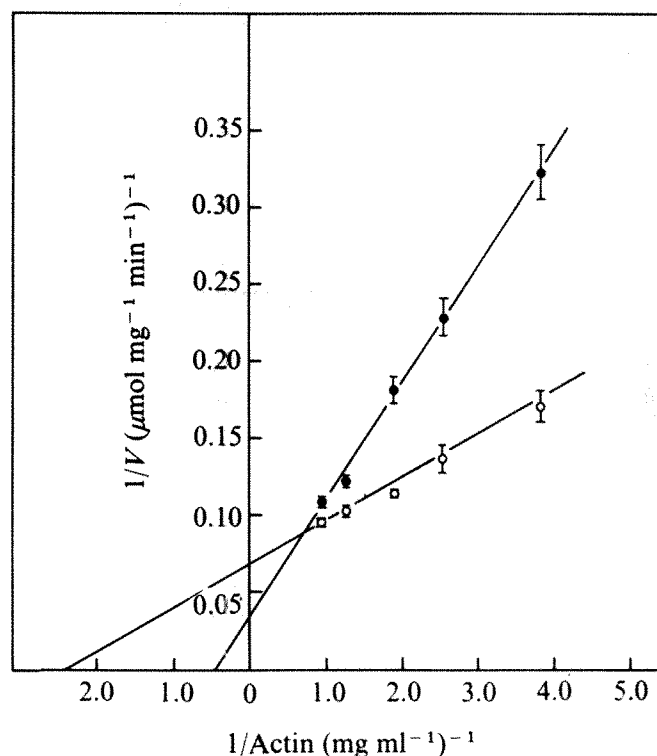


Fig. 3 Lineweaver-Burk plot of actin-activated ATPase activity for S-1(A1) (○) and S-1(A2) (●). The assay mixture contained 6 mM KCl, 1.5 mM MgCl₂ and 1.0 mM ATP, and activities were determined in a pH-stat at pH 8.0, 25 °C. The error bars show standard deviations, with at least five determinations at each actin concentration.

We show here that isoenzymes of S-1 obtained by chymotryptic digestion of filamentous myosin, can be separated to give homogeneous S-1 fractions. Steady state ATPase activity measurements showed no significant differences in the absence of actin, while in its presence there was a twofold difference in the V_{max} obtained from Lineweaver-Burk plots. This kinetic variation may reflect a true distinction in the mode of interaction of these different myosin heads with actin, which might be dependent on the presence of different alkali light chains, but it could have arisen as a result of the proteolysis procedures or during purification. Experiments are in progress to explore this. Nevertheless, the results suggest that the structural heterogeneity determines kinetic differences between these S-1 species. It is now important to establish whether the heavy meromyosins

produced by chymotryptic cleavage can be separated, since this would provide unequivocal evidence for the existence of myosin isoenzymes.

The importance of these studies is that using chymotrypsin, homogeneous S-1 preparations can be obtained for the first time. Furthermore, since the specificity of this enzyme is for aromatic residues, which are frequently buried and inaccessible to solvent, this enzyme provides a particularly sensitive probe for the susceptible regions of the myosin molecule: digestion can be modified not only by the aggregation state of myosin but also by its ionic environment, since in the presence of divalent cations (magnesium or calcium), HMM is produced even at low ionic strength (our unpublished observations).

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Induction of chromosome changes in Chinese hamster cells by exposure to asbestos fibres

THE occurrence of pleural and peritoneal mesotheliomata is linked with the inhalation of asbestos fibres^{1,2}. Experimentally, mesotheliomata have been induced by the intrapleural inoculation of asbestos fibres into rats³. These findings suggest that

the incorporation of the fibres disturbs processes at the cellular level. The possibility that they induce chromosome abnormalities was indicated by V. Timbrell (personal communication) and we have investigated it further using cultured Chinese hamster cells.

The cells were derived by cloning from a parental line CHO-K1 (American Type Culture Collection), a line which has previously been used in evaluating induced chromosome changes⁴. They were seeded at equal densities in 10 culture vessels containing Iwakata and Grace's modification of McCoy's 5a culture medium⁵ (foetal bovine serum level 10%). Pairs of flasks, with the exception of two unexposed control cultures, contained one of the following dusts at a concentration of 0.01 mg ml⁻¹, SFA chrysotile asbestos; UICC crocidolite asbestos; glassfibre and glass powder. These dusts have been described elsewhere⁶. The flasks were agitated to mix the cells and dusts thoroughly and after 48 h the cells from one member of each pair were collected, metaphase spreads being obtained by the standard procedure. Measurements of plating efficiency and viability (by the Trypan blue method) were made for each culture at the time of collection. Five days after seeding, the remaining cultures were processed in the same way. The slides were stained by the trypsin/Leishman banding method of Seabright⁶ and 100 suitable metaphases from each culture were studied.

The experiment was then repeated with the notable difference that the cells were treated after culture by a method of *in situ* fixation (to be published elsewhere) which avoided both mechanical handling (that is, centrifugation) and the use of proteolytic enzymes for cell detachment.

The results are summarised in Table 1 and show that karyotypic alterations were largely restricted to the cells exposed to asbestos. Prolonging the exposure period from 48 h to 5 d seems to have had little effect on the incidence of abnormalities, with the possible exception of the 5-d exposed SFA chrysotile group in which there was an increased trend toward cells with fragments. In this context, it is noteworthy that additional experiments in which asbestos fibres were preincubated in culture medium for a short period before coming into contact with cells revealed a reduced incidence of karyotypic abnormality, particularly in the case of the group exposed to UICC crocidolite.

This could indicate that components of the culture medium

Table 1 Effects of different treatments on chromosomes

	48 h exposure				
	SFA chrysotile asbestos	UICC crocidolite asbestos	Glassfibre	Glass powder	Unexposed controls
Polyploids	30 (33)	21 (24)	4 (3)	3 (5)	4 (4)
Cells with fragments	14 (13)	11 (15)	0 (0)	0 (0)	0 (0)
Cells with other chromosomal changes	28 (21)	32 (20)	0 (1)	0 (0)	0 (0)
% Abnormal cells	65 (56)	59 (55)	4 (4)	3 (5)	4 (4)
(100 cells scored from each culture)					
	5 d exposure				
	SFA chrysotile asbestos	UICC crocidolite asbestos	Glassfibre	Glass powder	Unexposed controls
Polyploids	21 (25)	25 (30)	7 (4)	5 (4)	2 (5)
Cells with fragments	34 (31)	14 (10)	0 (0)	0 (0)	0 (0)
Cells with other chromosomal changes	25 (19)	23 (30)	1 (0)	0 (0)	0 (1)
% Abnormal cells	79 (73)	53 (61)	8 (4)	5 (4)	2 (6)
(100 cells scored from each culture)					

The numbers in parentheses refer to results obtained by *in situ* fixation. The categories scored were not mutually exclusive. All the populations showed plating efficiencies > 89% and viabilities > 85% at the times of collection.

(for example, serum protein) interact with asbestos to produce alterations in the effective dose and exposure period within the experiments, such that after a fixed short period fibres were rendered ineffectual with regard to the production of genetic damage. This type of consideration may help to explain the similarity between the 48-h and 5-d exposure data.

The polyploid cells identified were largely cells with twice the modal number of chromosomes in the 48-h exposed groups and more than twice the modal number in the cultures receiving 5-d exposures. The cells classified with fragments (Table 1 and Fig. 2) included many changes at both the chromatid and chromosomal levels; chromatid breaks and paired isodiametric fragments (double minutes) as shown in Fig. 2b. In addition, chromosome type breaks and rearrangements were identified in some cells from the asbestos-treated cultures (Table 1 and Fig. 1), but not from the glass-treated or unexposed controls. The possibility that these may represent the selection of pre-existing clones is being examined in further experiments although this is not supported by ancillary evidence.

The inclusion of fibreglass and glass powder in the experiment as presumptively inert control dusts was prompted by the finding that, in contrast to asbestos fibres, neither gives rise to mesotheliomata when inoculated intrapleurally into rats³. It is noteworthy that polyploids were for the most part the only exceptional cells identified in the cultures exposed to these two dusts and that their incidence was no greater than in the unexposed controls. In the absence of other changes in the glass-exposed cultures it seems unlikely that the chromosomal abnormalities seen in the cells exposed to asbestos arose as a result of the mechanical handling (centrifugation) that followed cell culture. This is further confirmed by the results obtained

Fig. 1 a, Karyotype of unexposed cell. b, karyotype of cell exposed for 5 d to UICC crocidolite asbestos. Arrows indicate chromosomes that have undergone pericentric inversion. Chromosome 16 has broken in the region of its centromere and marker chromosome 20 is absent.

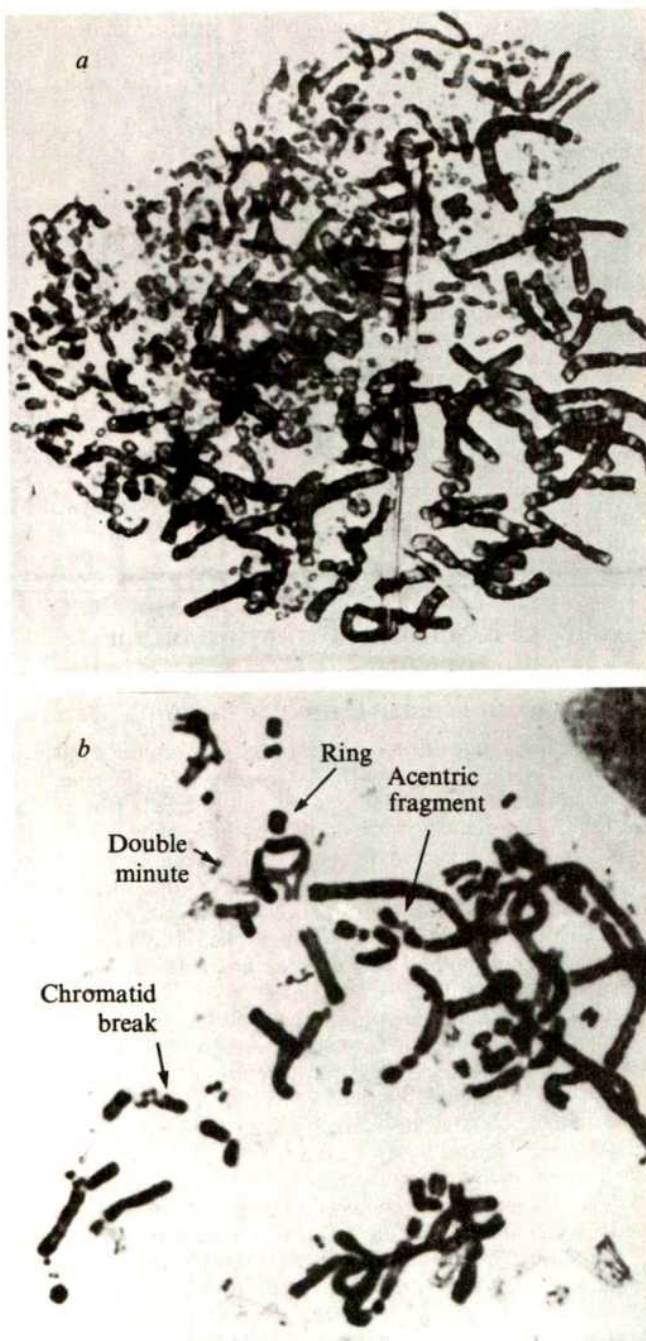
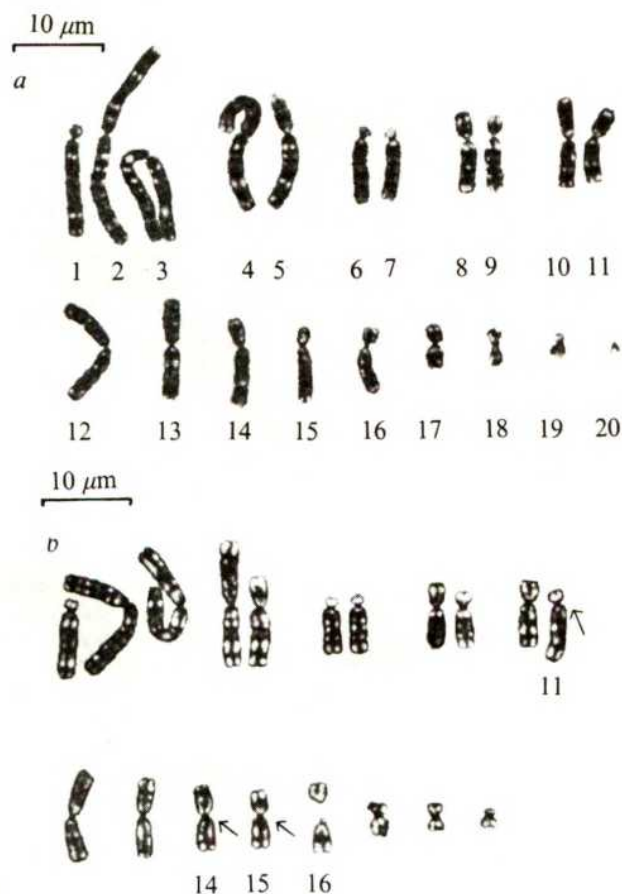


Fig. 2 a, Cell derived from a culture that had been exposed to UICC crocidolite asbestos for 5 d, showing multiple fragmentation in a situation of high ploidy. A fibre is seen at the centre of the field. b, Chromatid breaks and multiple aberrations in a cell derived from a culture exposed to UICC crocidolite asbestos for 5 d.

using an *in situ* fixation method (Table 1) which correlate closely with those obtained using standard procedures, although the method avoids both centrifugation and the use of proteolytic enzymes for cell detachment.

We conclude that multiple abnormalities at both the chromatid and chromosomal levels were produced in Chinese hamster cells as a result of the inclusion of asbestos fibre in their culture regime. Furthermore, these results, which were specifically produced by asbestos rather than the presumptively inert glass dusts, seemed to be largely unaffected by prolonging the exposure period. This preliminary detection of a mammalian cell response to asbestos *in vitro* forms the first stage of the development of an *in vitro* screening test for asbestos and other carcinogenic materials. In this it is envisaged that primary cells

will be cloned for specific chromosomal abnormalities and then introduced into laboratory animals to ascertain whether or not they induce tumours *in vivo*.

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Cyclic AMP-mediated transformation of rat cells transformed by temperature-sensitive mouse sarcoma virus

SEVERAL lines of evidence suggest that adenosine 3',5'-cyclic monophosphate (cyclic AMP) is involved in the regulation of growth and other properties of cultured cells (for review, see ref. 1). Specifically, various reports have shown a correlation between the abnormal growth and morphological properties of virus-transformed cells and low levels of cyclic AMP^{2,3}. Treatment of these transformed cells with cyclic AMP, cyclic AMP analogues, or agents that raise cyclic AMP levels restores growth and morphological properties characteristic of normal cells⁴⁻⁷. The use of cells transformed by temperature-sensitive (ts) mutants of Rous sarcoma virus (RSV) or mouse sarcoma virus (MSV)^{8,9} as well as ts host cell mutants^{10,11} strengthened the evidence relating viral transformation and cyclic AMP levels. Other evidence obtained in similar virally transformed cell systems, however, has failed to confirm the inverse relationship between cyclic AMP levels and cell growth rate and transformation by RNA and DNA tumour viruses¹²⁻¹⁵. We now report that the exogenous addition of cyclic AMP mediates the morphological transformation of rat cells transformed by a ts mutant of MSV at the non-permissive temperature. Correspondingly, expression of the transformed phenotype by cells grown at the permissive temperature is associated with increased levels of endogenous cyclic AMP.

The cell lines used have been described before^{16,17} and included: (1) normal rat kidney (NRK) cells, (2) NRK cells transformed and productively infected with the Moloney sarcoma-leukaemia virus (NRK(MSV-MLV)), and (3) NRK cells transformed by a cold-sensitive mutant of MSV (NRK(MSV-1b)). The NRK(MSV-1b) cells express the transformed phenotype at the permissive temperature (39 °C) but appear phenotypically normal at the non-permissive temperature (33 °C). Cell lines other than NRK(MSV-1b) were routinely grown at 36 °C.

Preliminary experiments to investigate the effect of cyclic AMP on the morphological phenotype of uninfected and MSV-transformed NRK cells, demonstrated that 0.4 mM 8-bromo-cyclic AMP (8-Br-cyclic AMP) or 0.4 mM N⁶,O^{2'}-dibutyryl cyclic AMP (db-cyclic AMP) in combination with 1.0 mM theophylline were most effective in inducing the morphological changes presented in Fig. 1. The morphology of NRK(MSV-1b) grown at the non-permissive temperature (Fig. 1a) was changed perceptively after exposure to 8-Br-cyclic AMP or db-cyclic AMP (Fig. 1b). The effect was to change the morphological phenotype of NRK(MSV-1b) cells

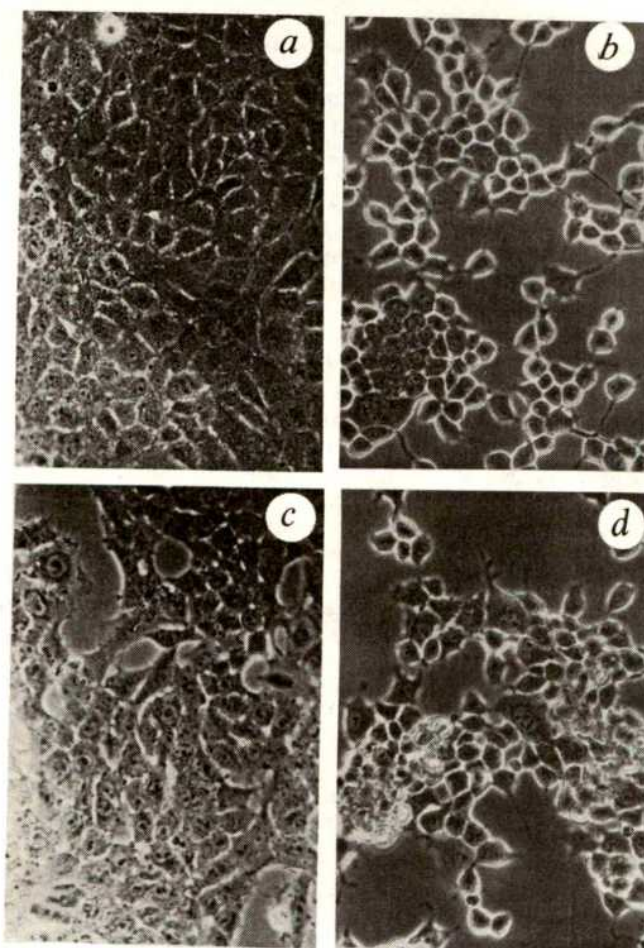


Fig. 1 Effect of cyclic AMP on the morphology of NRK(MSV-1b) cells. NRK(MSV-1b) cells were seeded at 1×10^6 per 60-mm Petri dish and incubated at either 33 or 39 °C for 3–4 d in Eagle's minimal essential medium (MEM) supplemented with 10% foetal calf serum (v/v). They were then exposed to 0.4 mM db-cyclic AMP plus 1.0 mM theophylline or 0.4 mM 8-Br-cyclic AMP without replacing the growth medium. Twenty-four hours later photomicrographs were made with a phase-contrast microscope and were magnified $\times 190$. Solutions of cyclic AMP, cyclic AMP analogues, other cyclic nucleotides, adenosine compounds and theophylline in growth medium were prepared and Millipore-filtered (0.45 μ m) immediately before use. *a*, NRK(MSV-1b) cells grown at the non-permissive temperature, 33 °C, and mock-treated with growth medium; *b*, NRK(MSV-1b) cells grown at 33 °C and exposed to 0.4 mM db-cyclic AMP plus 1.0 mM theophylline for 24 h; *c*, cells treated as in *b*, reversed at 24 h by replacing the medium with growth medium, and photographed 2 h later; *d*, NRK(MSV-1b) cells grown at the permissive temperature, 39 °C, and either mock-treated with growth medium, or exposed to 0.4 mM db-cyclic AMP plus 1.0 mM theophylline for 24 h. Identical results were obtained using 8-Br-cyclic AMP in place of db-cyclic AMP and theophylline.

which at the non-permissive temperature, 33 °C, appear flattened and poorly refractile to a highly refractile, rounded, circular morphology, characteristic of NRK(MSV-1b) cells grown at the temperature (39 °C) permissive for expression of transformation (Fig. 1d). The morphological change was obvious within 10 min after exposure to 8-Br-cyclic AMP and achieved maximum expression by 1 h. Using db-cyclic AMP plus theophylline, morphological changes were obvious within 3 h and achieved maximum expression by 24 h. In both cases, exposure of NRK(MSV-1b) cells at 33 °C to the cyclic AMP analogues resulted in morphological alterations of 100% of the cells, and the cells remained morphologically altered as long as additive was present (up to 72 h). Removal of the cyclic AMP analogues, however, resulted in a rapid reversal (within 1 h) from the transformed to the normal phenotype (Fig. 1c). Furthermore, the morphological changes induced

by cyclic AMP were apparently independent of the growth state of the NRK(MSV-1b) cells, since identical morphological responses were observed in sparse and confluent cultures. The addition of cyclic AMP analogues to NRK (MSV-1b) cells grown at the permissive temperature (39 °C) did not alter the existing transformed phenotype characterised by rounded cells that grew in clumps or clusters. The exogenous addition of cyclic AMP analogues to uninfected NRK cells and transformed NRK(MSV-MLV) cells provoked no morphological changes in these cells whether cultured at 33, 36 or 39 °C, indicating that the cyclic AMP-mediated morphological changes were specific for NRK cells transformed by the cold-sensitive MSV mutant.

The specificity of cyclic AMP or analogues of cyclic AMP in inducing morphological transformation of NRK(MSV-1b) cells grown at the non-permissive temperature was investigated. The induced morphological changes were specific for cyclic AMP and cyclic AMP analogues and potentiated by theophylline. Cyclic GMP alone had no effect on the morphology of NRK(MSV-1b) cells and was not antagonistic to the effects of the cyclic AMP analogues. Molecules related to cyclic AMP, such as 5'-adenosine monophosphate (5'-AMP), 2',3'-AMP, adenosine, and adenine had no effect on change of cell shape. Similarly, other cyclic nucleotides such as cytidine 3',5'-cyclic monophosphate (cyclic AMP), thymidine 3',5'-cyclic monophosphate (cyclic TMP), uridine 3',5'-cyclic monophosphate (cyclic UMP), as well as theophylline and butyrate had no effect on the morphological phenotype of the cells.

One prediction of the cyclic AMP-mediated morphological transformation event would propose that intracellular cyclic AMP levels should be lower in the phenotypically normal cold-sensitive transformants grown at the non-permissive temperature, whereas higher levels of cyclic AMP might be expected in the same cells grown at the permissive temperature where the transformed phenotype is expressed. To test this prediction, intracellular cyclic AMP content was measured by a standard radioimmunoassay (Collaborative Research) as previously described¹⁸. NRK(MSV-1b) cells grown under permissive conditions contained levels of cyclic AMP approximately three times higher than the same cells grown under non-permissive conditions (Table 1). Levels of cyclic AMP remained 50–80% higher in cells grown at the permissive relative to the non-permissive temperature during the course of the experiment. Reciprocal temperature shifts of NRK (MSV-1b) cells grown at 39 °C or 33 °C resulted in marked changes in cyclic AMP levels (Table 1). Cyclic AMP levels increased approximately threefold within 5 h after a shift from non-permissive to permissive temperature and reached a level 24 h later similar to that of cells grown at 39 °C. In shift-down experiments, cyclic AMP levels fell more slowly with time, resulting in a 50% decrease within 24 h after shifting from the permissive to non-permissive temperature. The slower drop in cyclic AMP levels contingent with shifting from 39 to 33 °C may reflect the time involved in turnover of the residual cyclic AMP pool. Alternatively, the rate at which cyclic AMP levels decrease or increase after temperature shift may be controlled by temperature-dependent adenylate cyclase activity.

During these experiments, uninfected NRK and transformed NRK(MSV-MLV) cell lines were included at random in the experimental design and their cyclic AMP levels are presented in Table 1. No significant difference in cyclic AMP levels was found for NRK and NRK(MSV-MLV) cell lines under the conditions described.

Our observations of higher levels of cyclic AMP in NRK (MSV-1b) cells grown at the temperature permissive for expression of the transformed phenotype relative to the same cells grown under conditions non-permissive for maintenance of transformation parallels the morphological changes that occur after addition of cyclic AMP or cyclic AMP analogues to these cells. Recently, we have examined fucosylglycolipid metabolism as a biochemical marker of transformation²⁰.

Table 1 Cyclic AMP levels in NRK cells transformed by temperature-sensitive MSV

Expt	Time	pmol cyclicAMP per mg protein				NRK NRK (MSV-MLV)	
		NRK(MSV-1b)					
		33 °C	33→39 °C	39 °C	39→33 °C		
1	0 h	28*	28	90	90		
	5 h	41	111	63	80		
	24 h	39	69	69	59		
2	5 d					61	51

In experiment 1 NRK(MSV-1b) cells were seeded at 1.5×10^5 cells per Petri dish and incubated at 33 or 39 °C. Forty-eight hours later, half of the dishes at 39 °C were shifted to 33 °C, and vice versa. At the time of temperature shift (0 time), 5 h and 24 h later cyclic AMP content was determined. Medium was aspirated from the cultures, the cells were washed once with cold Gibco Solution A, and the cells were frozen by placing the Petri dishes on dry ice. The cultures were then overlaid with 1.0 ml of cold 6% trichloroacetic acid, and the cells were scraped off the dishes with a rubber policeman. The suspension was centrifuged at 10,000g for 15 min at 4 °C. The precipitate was assayed for protein by the Lowry method¹⁹, and the supernatant was assayed for cyclic AMP. The supernatant was extracted three times with 5 ml of water-saturated petroleum ether. The aqueous phase was heated to 70–80 °C for 2 min and then frozen at –70 °C. In some cases the aqueous phase after extraction was lyophilised and the residue was dissolved in 0.25 M phosphate buffer, pH 7.0. Cyclic AMP was assayed using a standard radioimmunoassay (Collaborative Research) as previously described¹⁸. Recovery of cyclic AMP during extraction was above 95%. In experiment 2 NRK and NRK(MSV-MLV) cells were seeded at 1.5×10^5 cells per Petri dish, incubated at 36 °C, and assayed for intracellular cyclic AMP 5 d later, as described above. The values represent the mean of three independent experiments in which duplicate dishes were assayed in triplicate for cyclic AMP. *Values represent the mean of duplicate dishes. Cyclic AMP determinations were done in triplicate for each sample.

Concomitant with the morphological changes, fucosylglycolipid metabolism was altered markedly after addition of cyclic AMP to the cold-sensitive transformants grown at 33 °C (unpublished results of K.D.S., and S. M. Steiner). It thus seems that this system provides an important exception to mechanisms currently postulated for the role of cyclic AMP in the expression of the transformed state. A solution to the difference between the cyclic AMP-mediated morphological transformation of NRK(MSV-1b) cells and what is usually observed in comparison of cyclic AMP levels and effects in transformed and non-transformed cells awaits the elucidation of the nature of the cold-sensitive transforming function present in cells transformed by the MSV mutant. Perhaps more importantly, the rapid acquisition of the transformed phenotype after exposure to cyclic AMP provides a useful model for studying the control of viral gene expression and should aid in the identification and characterisation of viral gene products responsible for malignant transformation.

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Genetic heterogeneity in GM1-gangliosidosis

GM1-GANGLIOSIDOSIS is an inherited lysosomal storage disease which is due to a deficiency of the acid hydrolase GM1- β -galactosidase¹. During the past few years several clinical variants have been described²⁻⁶ that differ in time of onset of symptoms, involvement of visceral organs or skeletal tissue and in the degree of neuronal and mental deterioration. Some of these variants have been related to different properties of the deficient β -galactosidases^{5,7} but the significance of the experimental data⁸ has been questioned. Several investigators have speculated on the genetic background of the different variants^{2,8,9} but no experimental evidence has been provided to support the hypotheses.

Using somatic cell hybridisation techniques¹⁰⁻¹² we have investigated whether different gene mutations are involved in clinical variants of GM1-gangliosidosis. Fibroblasts from a patient with the infantile type 1 and the juvenile type 2 (refs 1-3), and from the type 3 patient without mental retardation described by Pinsky *et al.*⁵ were fused with cells from an adult (type 4) patient reported by our group⁶. Two days after hybridisation the occurrence of genetic complementation was tested by β -galactosidase assays with both synthetic and natural substrates. These experiments showed that the gene mutation in types 1 and 2 is different from that in types 3 and 4. Enzyme assays performed in single heterokaryons containing the genomes of type 1 and type 4 cells showed that genetic complementation results in complete restoration of normal enzyme activity.

Normal skin fibroblasts and cells from patients with the different types of GM1-gangliosidosis were grown in Ham F10 medium supplemented with 10% foetal calf serum and antibiotics. In homogenates of fibroblasts from patients with the type 1 or type 2 variant, β -galactosidase activity was less than 1% of control values when measured with the 4-methylumbelliferyl (MU) substrate. Homogenates of fibroblasts from patients with type 3 or type 4 GM1-gangliosidosis had a residual activity of 15-20%. In type 4 cells a 5% and 8% residual activity with the natural substrates GM1-ganglioside and asialofetuin was found by Dr J. S. O'Brien (Department of Neurosciences, University of California, La Jolla).

Cells from the adult type 4 patient were hybridised with those from the other three variants and each cell strain was also fused with itself (parental fusion) as a control. The results of β -galactosidase assays with synthetic substrate are shown in Fig. 1. Levels of activity in unfused cells were similar to those in the parental fusions, which indicated that somatic cell hybridisation did not affect enzyme activity. The β -galactosidase activities in fusions of type 1 \times type 2 and of type 3 \times type 4 cells remain unchanged relative to the average level expected in a mixture of equal numbers of parental cells. Fusion of type 1 \times type 4 cells, however, resulted in a six- to sevenfold increase in enzyme activity. Also there was a markedly increased enzyme activity in fusions of type 4 \times type 2 cells.

Similar results were obtained when the β -galactosidase activity was measured with the natural substrate GM1-

β -galactosidase activity
($\times 10^{-9}$ mol per h
per mg protein)

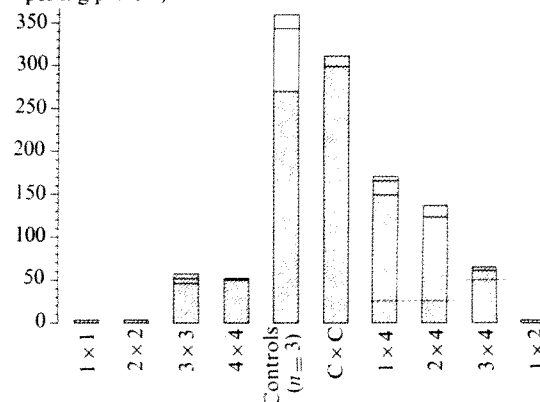


Fig. 1 β -Galactosidase assays on cell homogenates after fusion of cultured fibroblasts from patients with different variants of GM1-gangliosidosis. 2×10^6 cells from each strain were hybridised with inactivated Sendai virus¹³ under such conditions¹¹ that 50-80% of the nuclei were present in multinucleates. Two days after fusion cells were trypsinised, rinsed in saline and disrupted by sonication (2×30 s), and after protein analysis¹⁴ the β -galactosidase activity was assayed with 1 mM 4-methylumbelliferyl- β -D-galactopyranoside, pH 4.2, as described previously¹⁵. Independent experiments with the same cell strain; as controls three different normal strains were used; ———, expected enzyme activity if no complementation occurs, that is the mean enzyme activity as a result of mixing equal numbers of both parental cells.

ganglioside (Table 1). Conditions for cell cultivation, fusion, collection and protein determination were identical to those described in Fig. 1. Whereas no enzyme activity was detected after type 1 \times type 2 fusion a marked increase in GM1- β -galactosidase activity was observed after hybridisation of type 4 \times type 1 cells. An additional fusion experiment of type 4 \times type 1 cells was carried out with inactivated serum in the culture medium. This will inhibit proliferation of the unfused parental cells and thus the proportion of heterokaryons will be higher than if normal serum is used. This is reflected in the higher β -galactosidase activities in the last type 4 \times type 1 fusion shown in Table 1.

These results showed that genetic complementation had occurred in the fusion between type 4 cells and type 1 or type 2 cells. Thus the adult form of GM1 gangliosidosis (type 4 variant) is caused by a different gene mutation than is present in the types 1 or 2 variants. Since there was no complementation after fusion of type 4 \times type 3 cells these variants might be identical or determined by allelic mutations, as is the case for types 1 and 2 GM1-gangliosidosis¹⁷.

Although complementation could be detected by enzyme analyses in homogenates of heterokaryons it is not possible to evaluate the degree of restoration of activity since the proportions of nuclei from each parental cell present in the multi-

Table 1 β -Galactosidase assays using natural substrate after somatic cell hybridisation of different GM1-gangliosidosis variants

	Enzyme activity ($\times 10^{-9}$ mol per h per mg protein)	
	GM1- β -galactosidase	4-MU- β -galactosidase
Type 1 \times type 1	3.5	1.8
Type 2 \times type 2	4.3	4.0
Type 4 \times type 4	10.5	13.4
Type 1 \times type 2	<1	<1
Type 1 \times type 4	44.1	38.6
Type 1 \times type 4*	99.8	75.3
Control	193.8	246.7

*After fusion, cells were grown on medium with 10% foetal calf serum that had been treated with alkali (pH 10.7) for 3 h at 37 $^{\circ}$ C to destroy enzyme activity in the medium. Conditions of cell culture, hybridisation and collection were similar to those described earlier. GM1-galactoside- β -galactosidase activity was measured according to Ho *et al.*¹⁶.

nucleates are unknown. To solve this problem microchemical techniques were developed which facilitated quantitative enzyme assays in individual binuclear heterokaryons^{15,18}. Fibroblasts from the adult patient with type 4 GM1-gangliosidosis were hybridised with those from a type 1 variant under such conditions that about 8% of the hybrid cells are binucleate¹⁵. After fusion cells were grown for 24 h in a Falcon flask, they were then trypsinised and 2×10^4 – 4×10^4 cells were seeded in a dish with a thin plastic bottom. After 24–48 h of subsequent cultivation these dishes were frozen and freeze-dried immediately and small pieces of plastic each containing one binuclear cell or one non-fused mononucleate cell were dissected under microscopic control. β -Galactosidase assays in individual cells were carried out with synthetic substrate in submicrolitre volumes and fluorescence measurements were made in a microspectrofluorometer as described earlier^{15,18}.

The distribution of β -galactosidase activity in individual unfused control fibroblasts is shown in Fig. 2a. Results from three different cell strains were pooled, but also in cells from each cell strain there was a considerable variation of activity in the range 4×10^{-14} – 40×10^{-14} mol h⁻¹. In considering these data it should be realised that the enzyme activity is expressed per cell and that the total cellular dry mass and protein content in individual fibroblasts also show marked variations^{18,19}.

The mean β -galactosidase activity in control cell strains varied from 19×10^{-14} to 26×10^{-14} mol h⁻¹. Earlier experiments¹⁷ showed that the level of activity in parental fusions of control fibroblasts was approximately twice that of mononucleates. No β -galactosidase activity could be detected in

individual binuclear cells after parental fusions of type 1 cells while in parental fusions of type 4 cells mean activities of 3×10^{-14} and 5×10^{-14} mol h⁻¹ were found in two experiments; this is 10–20% of the activity in parental fusions of control fibroblasts (Fig. 2b).

After fusion of type 4 \times type 1 cells in 21% of the binucleates no β -galactosidase activity could be detected; these probably represent fusions of type 1 \times type 1 cells. In 27% of the binuclear cells the enzyme activity was similar to that in parental fusions of type 4 cells. But in 52% of the binuclear heterokaryons β -galactosidase activity was in the range 8×10^{-14} to 40×10^{-14} mol h⁻¹ (Fig. 2c). This is greater than in either of the parental cells before or after fusion and the range of activity is similar to that in mononuclear control fibroblasts. Thus genetic complementation after fusion of type 4 \times type 1 cells results in restoration of β -galactosidase activity to normal control levels.

The mechanism responsible for the genetic complementation observed after fusion of type 4 cells with type 1 or type 2 cells is not yet understood. In human liver two forms of GM1-ganglioside- β -galactosidases (A and B) have been identified^{16,20} both of which are deficient in GM1-gangliosidosis²⁰. It has been proposed that the A form of β -galactosidase consists of a single polypeptide chain²¹ and that the B form is a multimeric aggregate of the A monomer⁹. On this basis the results of our hybridisation studies would indicate the occurrence of intragenic complementation. In a recent review, O'Brien⁹ does not exclude the possibility that the B form of β -galactosidase also contains amino acid sequences which are not common to the A form. In that case the complementation in type 4 \times type 1 or 2 heterokaryons could still be intergenic. A third possibility is that the gene mutation in type 4 GM1-gangliosidosis involves a regulator whereas the mutations in type 1 and type 2 are structural mutations. The latter is supported by immunological studies^{9,22} which revealed the presence of cross-reactive material in cells from all patients tested with type 1 and type 2 GM1-gangliosidosis. Preliminary analyses of the deficient β -galactosidase in type 4 cells and cocultivation studies are suggestive for a regulator mutation in the adult type 4 GM1-gangliosidosis. Further investigations on the immunological and biochemical properties of the deficient enzyme and of the restored enzyme activity after genetic complementation are required to prove this hypothesis.

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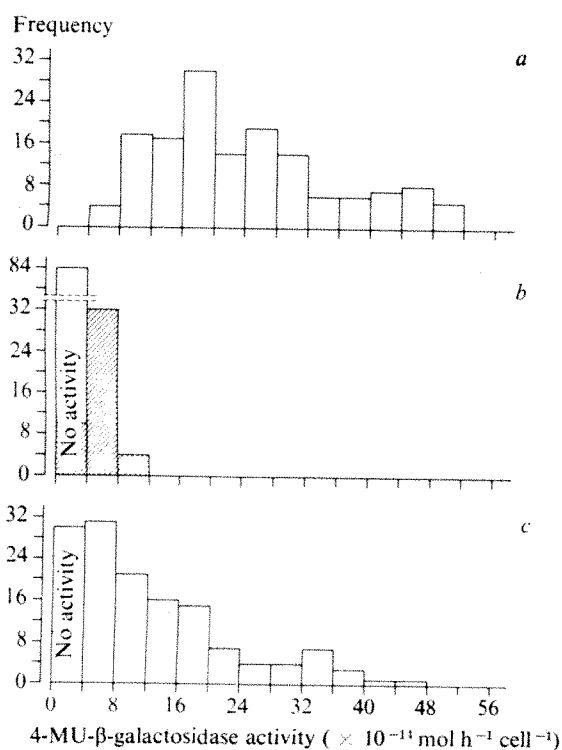


Fig. 2 β -Galactosidase assays in individual heterokaryons after fusion of type 4 \times type 1 variants of GM1-gangliosidosis. β -Galactosidase activity was measured by incubating individual cells in 0.2 μ l 1 mM 4-methylumbelliferyl- β -D-galactopyranoside in 0.1 M-acetate buffer (pH 4.2) containing 0.02% (w/v) bovine albumin. Incubation was carried out during 2 h at 37 °C under paraffin oil using Lowry's "oil well technique"^{15,18}; after addition of 1 μ l 0.5 M carbonate buffer (pH 10.7) the fluorescence was measured in 1-mm glass capillaries with a Leitz microspectrofluorometer^{15,18} (objective 2.5 pl). a, Distribution of enzyme activities in non fused mononuclear control fibroblasts as measured in three different cell strains; b, enzyme activities in individual binuclear cells after parental fusion of type 1 \times 1 (open columns) and type 4 \times 4 (hatched columns) cells each measured in two independent experiments; c, enzyme activities in binuclear heterokaryons after fusion of type 4 \times 1 cells as measured in three independent experiments.

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Influence of sexual dimorphism on foetal and placental weights in the rat

THE male is heavier than the female in the adults of most mammalian species. This trend is apparent at birth in humans¹ and in many other mammals including the sheep, goat, cow, rat and monkey². Little is known however, of the developmental basis for these birth weight differences. We have found that, one day before birth, male rat foetuses are 5% heavier than female foetuses, yet there seems to be no difference in the weights of their placentae.

Heavier foetuses are generally associated with heavier placentae³. This could be a causal relationship, where foetal growth is limited by placental function, as reflected by placental weight, or it may merely be a consequence of the common origin of the foetus and the foetal component of the placenta³. Whatever the reason, it might be expected that male foetuses would have heavier placentae than female foetuses. Here we examine the influence of sex on foetal and placental weights in the rat as part of a general study on the control of placental development and function. We have also tested the hypotheses that the sex of a conceptus has a general systemic effect on the growth of all other conceptuses in the litter and a local effect on its immediate neighbours since there is some evidence of similar effects in man⁴.

Thirty-four albino rats with a mean weight at mating of 238 ± 3 g (mean \pm s.e.) were used. The day spermatozoa were found in a vaginal smear was called day 1 of gestation. On day 22, the day before expected parturition, each rat was killed with an overdose of sodium pentobarbitone, its uterus removed and the number and position of all implantation sites recorded. The uterus was then opened and each conceptus examined in detail. Only 'live' foetuses, which moved in response to pressure, were included in the results. The sex of each foetus was determined by the distance between the anus and the genital tubercle (about 2 mm in males and 1 mm in females). The internal genitalia were examined in 25 of the foetuses and were always found to agree with the external morphology. The foetal membranes and umbilical cord were removed from each conceptus; the foetus and placenta were wiped dry and weighed separately.

There were 9.9 ± 0.6 live foetuses per litter: 49.7% were males. Thirty-four conceptuses, about one per litter, died at various stages before day 22. The mean foetal weight per litter was 4.598 ± 0.056 g, the mean placental weight was 0.463 ± 0.006 g and the mean foetal weight : placental weight ratio was 10.049 ± 0.220 .

In the rat, conceptuses in the middle of both uterine horns tend to be heavier than those at either end⁵. Any selective positioning of male and female conceptuses along the horn

could have caused this trend or alternatively, have affected any appraisal of differences in male and female weights. No selective positioning was apparent however, since the numbers of males and females were respectively 27 and 26 at the ovarian end, 20 and 30 at the middle and 20 and 29 at the vaginal end of the horns. Horns containing foetuses of one sex only were excluded.

Sex differences in conceptus weights were determined in each horn to avoid between rat and between horn variation. Male foetuses were found to be 5.4% or 0.247 ± 0.030 g heavier ($P < 0.001$, $n = 55$) than female foetuses. The placentae of male foetuses were however, only 1.3% or 0.006 ± 0.008 g heavier (not significant). The foetal weight : placental weight ratio was thus higher (5.2%, $P < 0.005$) in males than in females.

The systemic effect of the number of males and the number of females in the litter on mean foetal and placental weights was examined by analysis of covariance. Larger litters, irrespective of sex, had smaller placentae $r = -0.528$ ($P < 0.01$, $n = 34$). At equal litter sizes however, there was no apparent difference in the effect of the number of males or the number of females on mean placental or foetal weights of either sex.

The local effect on each foetus and placenta of the sex of its immediate neighbours was assessed by classifying each foetus as having either 0, 1 or 2 neighbours of the opposite sex and then comparing foetal and placental weights between these classes. Comparisons were made within each horn to avoid between horn variation, consequently the number of horns with examples of two or more of these classes was reduced. Conceptuses were excluded if their immediate neighbour was dead, since the dead conceptus could not be sexed and may, itself, have had a local effect.

Male conceptuses seemed to be little influenced by the sex of their neighbour although male foetuses and placentae with two female neighbours were 3.8% and 4.5% heavier (not significant, $n = 7$) than those without female neighbours. No other male class comparisons yielded differences of greater than 0.7%. There was more indication that female conceptuses were influenced by the sex of their neighbour. Female foetuses with one male neighbour were 2.9% heavier ($P < 0.05$, $n = 27$) than those without male neighbours. Placentae of female foetuses with two male neighbours were 6.5% ($P < 0.05$, $n = 9$) and 8.6% (not significant, $n = 4$) lighter than those with one or no male neighbours respectively. No other female class comparisons yielded differences of greater than 1.3%.

The principal finding to emerge from the above results was that male foetuses are about 5.4% heavier than females but their placental weights are remarkably uniform. Similar observations have been reported in the monkey³. These findings raise a number of questions about the developmental basis of sexual dimorphism.

Sexual differences in growth rates of conceptuses, either by cellular enlargement or by multiplication, can be attributed directly to a genotypic difference and indirectly to various environmental differences evoked by the genotype. It has been shown that even at the blastocyst stage, the genotype of the conceptus can affect its own growth rate⁶. In this way the XX or XY chromosome complement may have a differential effect on the growth rate of cells, irrespective of environmental influences. While there is no evidence as yet for such a genetic effect, male-female weight differences seem to depend on more than just hypothalamic or gonadal hormones since weight differences are evident even in anencephalic foetuses⁷. The latter would be expected to have low, if any, levels of these hormones, at least in the second half of gestation⁸. On the other hand, if the sex chromosome complement intrinsically affects cell growth or division in the foetus, it is difficult to explain why it did not affect the placenta, an organ largely composed of foetal tissue.

Environmental factors which might influence male-female weight differences include the interaction of the foetus with its mother and gonadal hormones. The degree of antigenic interaction of mother and foetus in mice can influence placental and subsequently foetal size⁹. Since male foetuses possess the

greater antigenic dissimilarity they might be expected to have heavier placentae⁴. Our placental weight findings would however, seem to discount this hypothesis. Gonadal hormones are often used to explain sexual dimorphism. They do affect the differentiation of the reproductive tract of the foetus, so they might also affect its growth rate. The evidence of the anencephalic children however, tends to oppose this idea. In addition, if male gonadal hormones, generally considered to be anabolic, affect foetal growth, why do they not affect placental growth? Perhaps the placenta is not a target for gonadal hormones or, if it is, it may respond by improved efficiency rather than by weight. Differences in the efficiency of the placenta or of the foetus to use its placenta could mask any association of foetal and placental weights expected if foetal growth is indeed limited by placental function at the level of sexual dimorphism.

The possibility that each foetus, depending on its sex, releases or evokes circulating substances in its mother, that in turn affect the growth rates of other foetuses in the litter, was not supported by any evidence of systemic effects. On the other hand, it did seem that the sex of a foetus can influence the growth rates of its neighbours, presumably by releasing or evoking locally acting substances. Although this finding requires confirmation, there is good evidence that other hormones, notably prostaglandins, are produced in the uterus and have powerful local effects extending even to the ovary¹⁰.

In the rat and several other mammals, sexual dimorphism in relation to size is apparent even before birth. A closer examination of this phenomenon and its ramifications may well lead to a better understanding of the control of prenatal growth.

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Prolactin levels and duration of postpartum anoestrus in lactating ewes

THE length of the postpartum anoestrous period in sheep is related to the degree of mammary stimulation. If the ewe is milked by hand twice a day or 'dried off' immediately after parturition, the first oestrus occurs 30-40 d postpartum in the 'Préalpes du Sud' breed; if the ewe is nursing her lamb(s)—suckling 10-12 times per day—postpartum anoestrus lasts for 60-80 d (ref. 1). A similar phenomenon has been described in women; menses reappear within 10 weeks postpartum in 80% of women who are not breast feeding, but in only 10% of those who are². In both species suckling is accompanied by large discharges of oxytocin and prolactin; this reflex secretion of prolactin decreases as lactation progresses, and is almost nonexistent by the time of return of cyclical ovarian activity.

Amenorrhoea is also associated with high blood levels of

prolactin in women who are not breast feeding, and menses return after the prolactin levels have been lowered by hypophysectomy or administration of a prolactin-suppressing drug like 2-bromo- α -ergo-cryptine³.

These facts all suggest that prolactin has an antigonadal action^{4,5}; we have therefore studied ovarian and pituitary activity in lactating ewes after denervation of the mammary gland to inhibit the afferent arc of the suckling reflex. Denervation can be achieved by several means⁶. Bilateral section of dorsal pathways of the spinal cord at T₁₁, and bilateral sympathectomy and section of the perineal nerves results in a disappearance of the normal prolactin surge observed during milking or suckling. A similar effect is observed after the bilateral section of the afferent roots of lumbar nerves 1, 2, 3 and 4, lumbar sympathectomy, and section of the perineal nerves. Unilateral section of the afferent nerves from the mammary gland shows that the spinal reflex resulting in a prolactin surge after teat stimulation is ipsilateral (Fig. 1).

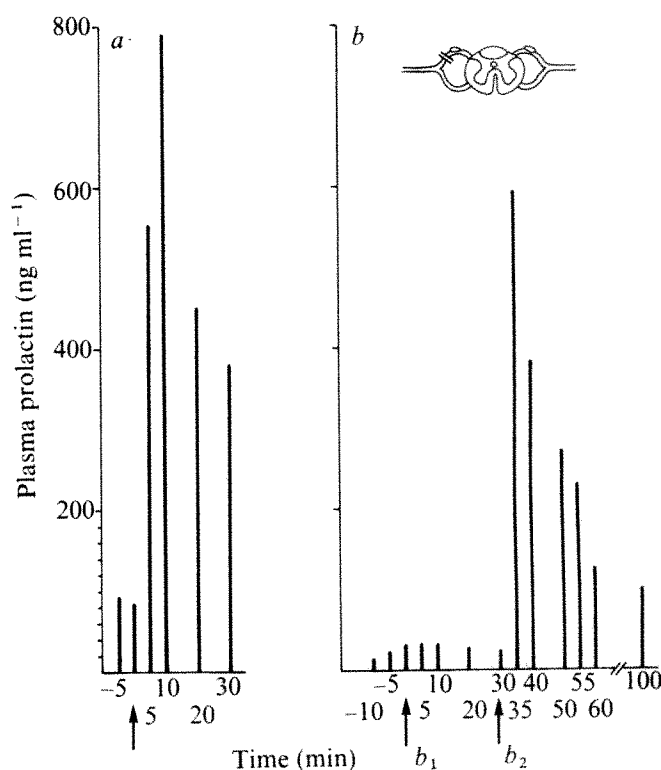


Fig. 1 Prolactin levels in suckled ewes before and after unilateral severance of lumbar nerves 1, 2, 3 and 4. Results expressed as ng ml⁻¹ (NIH, PS7). a, Reflex prolactin secretion triggered by suckling (arrow) in a normal ewe; b, after unilateral mammary denervation; b₁, disappearance of reflex prolactin secretion after ipsilateral teat stimulation (arrow); b₂, restoration of the reflex on contralateral teat stimulation (arrow).

All nervous connections between the mammary gland and the body were severed, including perivascular sympathectomy of the inguinal blood vessels, in five 'Préalpes du Sud' ewes during late pregnancy. After normal parturition, ewes were allowed to suckle their lambs, as were eight unoperated control ewes, which lambed at the same time. Blood samples were obtained twice daily for radioimmunoassay of prolactin and luteinising hormone (LH)^{7,8}, and oestrous behaviour was checked after blood sampling using a vasectomised ram. Laparoscopy was carried out on all the ewes between days 12-15, 25-31 and 40-50 postpartum to examine the state of the ovaries.

The normal prepartum surge of prolactin secretion and the

basal levels during lactation were unaffected in the denervated animals, but the suckling stimulus of the lambs was not followed by the normal reflex discharge of prolactin. As in previous experiments⁶, the growth of the lambs of these denervated ewes was normal.

In control animals, neither ovulation nor an LH peak was observed before 25 d *postpartum*; five of the eight animals exhibited silent ovulations preceded by an LH surge between days 25 and 50 *postpartum*, and only three of them came into oestrus during this period. In marked contrast, three of the five operated animals had ovulated without showing oestrus before day 14 *postpartum*, and all five animals had ovulated and shown oestrus before day 50.

These results establish that it is the suckling stimulus, and not lactation itself, that in some way suppresses the resumption of cyclical ovarian activity. In preliminary experiments we have been able to show that pharmacological suppression of prolactin secretion in suckling ewes with 2-bromo- α -ergocryptine (CB154, Sandoz) is also followed by an early restoration of cyclical ovarian activity⁹. Therefore it seems reasonable to conclude that it is the surges of prolactin secretion specifically induced by suckling that are in some way responsible for the suppression of ovarian activity during lactation.

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Structure of DNA molecules in yeast meiosis

THE nuclear DNA of the yeast *Saccharomyces cerevisiae* has been the subject of a detailed electron microscopic examination^{1,2}. These studies have revealed long, continuous molecules of lengths up to 355 μm , which correspond to the molecular weights of nuclear DNA estimated from sedimentation analysis in sucrose gradients. Several replication structures (bubbles) were observed along single molecules^{2,3}, as found in other eukaryotes^{4,5}. The most useful observations were made on selected replicating DNA³ and on DNA of mutants that were arrested just after the initiation of DNA synthesis⁶. We have started to study the structure of DNA molecules during meiosis because of its direct relevance to the process of recombination. Models to explain recombination (for review see ref. 7) are based mostly on genetic data from meiotic analysis, among which yeast tetrads occupy an important position. The various models have molecular implications which could best be tested by the direct examination and quantification of the interactions among DNA molecules, and between DNA and proteins, during meiosis.

Three unique features are expected to characterise meiotic DNA. First, the longer duration of the premeiotic DNA synthesis may be the result of the presence of fewer replicons than in the vegetative replication, as found in *Triturus*⁸. It has also been suggested that certain regions remain unreplicated during the premeiotic S period and are completed only during zygotene⁹. The second unique feature of meiotic DNA inter-

actions is pairing; the four double helix molecules that constitute the four chromatids of a bivalent (provided one accepts the unine structure of the chromatid⁹) must be paired intimately. The third feature, breakage and reunion between the paired molecules, is expected from the genetic data to occur 5–15 times along an average-sized bivalent of yeast¹⁰. This is the act of recombination itself, based probably on base pairing between antipolar single-stranded molecules from different chromatids. Depending on the model⁷, single-stranded regions either remain in the backbone of the DNA molecules (chromatids), and are later repaired by polymerase and ligase, or they remain projected from the double-stranded molecules and are later digested by exonuclease/ligase repair activity.

Vegetative cells of strain 419/1 that were labelled with ³H-6-uracil as described in the legend to Fig. 1, were transferred to sporulation medium (SPM) to give meiosis with kinetics

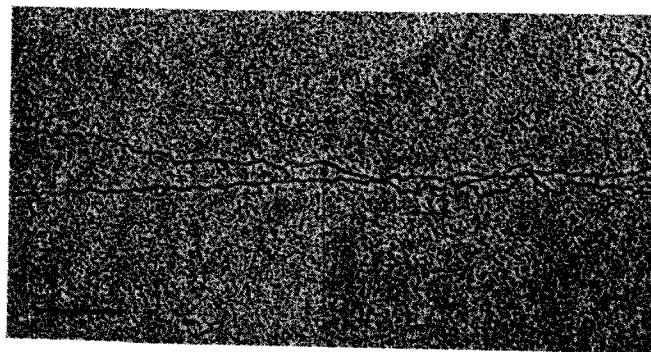


Fig. 1 Pairing between two DNA molecules. These molecules were paired over a length of 30 μm and were clearly connected to each other in at least two other places. The threads linking the two molecules here are probably single-stranded, at least over part of their length. DNA was prepared for electron microscopy by a modified Kleinschmidt technique¹⁴. The diploid yeast strain 419/1 is a *ura1/ura1* homozygous derivative (obtained after low-dose ultraviolet irradiation) of strain 419 (ref. 11). DNA was prepared from meiotic cells for equilibrium sedimentation analysis after they were grown overnight at 30 °C in PSP2 medium¹² supplemented with adenine (40 $\mu\text{g ml}^{-1}$), uracil (20 $\mu\text{M ml}^{-1}$) and ³H-6-uracil (3 $\mu\text{Ci ml}^{-1}$, specific activity of label 13.6 Ci mM^{-1}), to a titre of 10⁷ cells ml^{-1} and were washed and resuspended in SPM for 6.75 h. Spheroplasts were prepared from 5 ml of the culture by 30-min incubation with Glusulase (Endo Lab.) in spheroplasting buffer¹³, lysis was obtained following their transfer to a lysis buffer¹³, and the lysate was mixed with a saturated solution of CsCl, to a volume of 5 ml, with an average buoyant density of 1.690 g ml^{-1} . Density gradients were obtained in cellulose nitrate tubes following 60-h runs in the Spinco type 50 rotor at 35,000 r.p.m. The tubes were pierced and dripped into 35–40 tubes, from which samples were counted for radioactive label, following an overnight alkali treatment (NaOH 0.5 N) and TCA precipitation (BSA added as carrier). The nuclear DNA was separated from the mitochondrial DNA by about 12 fractions, the former giving a very sharp peak of 2–4 fractions, from which DNA was spread for the electron microscopy. Samples (10 μl) were taken from the nuclear peak fractions of the CsCl gradients. The DNA concentration in these fractions was estimated to be about 1–2 $\mu\text{g ml}^{-1}$. Each sample was added to 30 μl of a freshly prepared mixture, containing 0.1 mg ml^{-1} cytochrome c, 3 M ammonium acetate buffer (pH 6.8) and 50% formamide. After gentle agitation, 5 μl were withdrawn and spread, in a wax-coated depression plate, on a 1-ml hypophase containing 0.3 M ammonium acetate buffer (pH 6.8) and 10% formamide. A carbon-coated grid was used to pick up a small drop from the hypophase surface. The grid was then dehydrated in absolute ethyl alcohol and shadowed with platinum–palladium (80:20) at an angle of 10° in vacuum of 5×10^{-5} mmHg. A JEOL-JEE 4B high vacuum evaporator with a rotatory table (60 r.p.m.) was used for the shadowing. A JEOL 7A electron microscope was used, with 40- μm foil objective aperture and 80 kV accelerating voltage. Magnification was calibrated with a carbon grating replica, 28,800 lines per inch (Ernest F. Fullam, Inc., Schnectady, New York). DNA control standards were *Herpes simplex* type 1 DNA (mean length $54.3 \pm 0.3 \mu\text{m}$) and $\phi\text{X174 RF}$ DNA (mean length of $1.8 \pm 0.3 \mu\text{m}$) that were received from Y. Shlomai and A. Razin, respectively. Bar represents 0.5 μm .

slightly faster than in related strains^{11,12}: the premeiotic DNA synthesis took place between 4 and 7 h, the first meiotic division took place at about 8 h, recombination commitment (ADE colonies) was from 5 to 8 h and haploidisation was from 7 to 9 h. The short duration of each meiotic stage and the asynchrony in the culture (estimated to be 2–3 h) resulted in a notable degree of overlapping between the premeiotic replication and the early meiotic stages.

Vegetative DNA spreads, prepared from the nuclear peaks of CsCl gradients, appeared in the electron microscope to be similar in every respect to the molecules described by Petes *et al.*^{1–3}. Linear molecules were of various lengths up to 300–400 μm . A few molecules were in replication, with 'bubbles' ('eye-forms') of various lengths. We also observed small circular molecules, 2 μm in circumference, as described previously¹⁵. Some of these small molecules were supercoiled and a few were replicating, showing a closed, symmetrical, bacteria-like replicon.

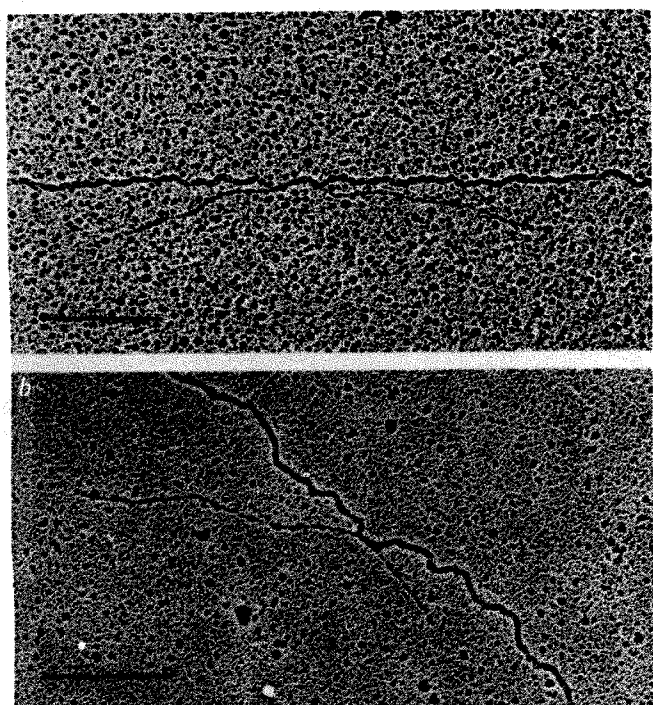


Fig. 2 Parts of linear double-stranded molecules with two single-stranded projections each. *a*, The lengths of the projections are 1.0 and 0.8 μm and the intermediate region (between the branching off points of the projections) is 0.2 μm ; *b*, the lengths of the projections are 1.16 and 0.28 μm and the intermediate region is 0.1 μm . Bars represent 0.5 μm .

Meiotic DNA spreads were prepared from samples taken at 5, 6, 6.75, 7.5 and 8 h in sporulation. At the two earliest times, we observed 'bubbles' of various sizes, including some much longer than those found in vegetative DNA. These molecules could, however, be paired molecules that were merged over short distances. As yet we find it difficult to resolve with certainty very long 'bubbles' from paired molecules. Paired molecules were also common in our preparations from 6 h onwards, including pairing arrangements of three and four molecules (see, for example, Fig. 1). Comparable pairing configurations were not observed in any of our vegetative DNA preparations.

The most characteristic feature of the meiotic DNA molecules were single-stranded projections observed quite commonly in the 6-h preparation and with higher frequency in the 6.75-h preparation. The projections usually occur in pairs (Fig. 2) and

sometimes they appear repeatedly along the same molecule. In most cases, the single-stranded projections seem quite convincingly to be an integral part of the main double-stranded molecule. We did not see such single-stranded projections in the vegetative DNA preparations, nor did we see them on the 2- μm circles in the meiotic DNA preparations. (The circles in the vegetative and meiotic preparations had a similar appearance.) In some preparations there seemed to be a certain affinity between the single-stranded projections of different molecules, resulting in complex configurations. At this preliminary stage, we cannot resolve these, or other complex molecules.

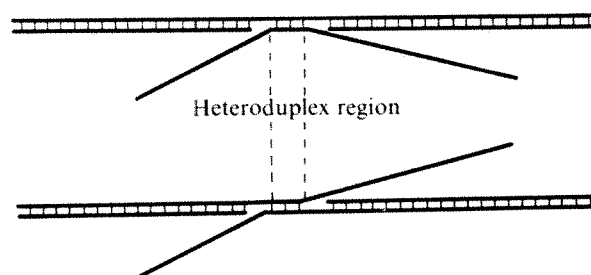


Fig. 3 Two possible interpretations of the molecule in Fig. 2a.

The pairs of single-stranded projections are most easily interpreted as the simplest recombination, or gene conversion, configurations outlined in Fig. 3. We believe these projections to be the result of genetic recombination, but we cannot exclude the possibility that they are intermediate structures towards the act of recombination. If, however, the former interpretation is correct, and Fig. 3 is taken at face value, the length of the heteroduplex region that resides between the two projections can be estimated. In Fig. 2a, this length is approximately 0.2 μm and in other pairs of projections the length of the intermediate region was found to be between 0.1 and 0.5 μm . The heteroduplex region in Fig. 2a is about 600 nucleotides long, and this corresponds well with the length estimated from genetic data on coconversion in yeast¹⁶.

Electron microscopic analysis of the structure of meiotic DNA can aid the understanding of genetic recombination at the molecular level and the choice between the various proposed models⁷. Our experience with this material, however, has convinced us that a systematic study of the variety of configurations encountered can only be made if accompanied by the use of mutants or treatments that accumulate intermediate configurations. As no true *rec⁻* mutants are available in yeast, we are now studying DNA structure in the *cde* mutants¹⁷, which block meiosis¹¹ at defined stages of DNA synthesis or nuclear division.

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Major mRNA species from spinach chloroplasts do not contain poly(A)

POLY(A) is a constituent of most animal cytoplasmic mRNAs^{1,2} and has been demonstrated in rapidly-labelled, polysomal RNA in plants^{3,4}. Verma *et al.*⁵ have shown that cellulase mRNA, isolated from auxin-treated pea epicotyls, contains poly(A). Although a significant proportion of eukaryotic polysomal mRNAs are known to lack poly(A)^{6–8}, the presence of poly(A) is still regarded as a characteristic feature of unfractionated preparations of eukaryotic mRNA. By contrast, poly(A) has not been detected in mRNA from prokaryotic cells⁹. Several reports^{9–11} have indicated that poly(A) is present in HeLa cell mitochondrial RNA, although Groot *et al.*¹² were unable to detect poly(A) in yeast mitochondrial RNA. Here we describe experiments in which we have assayed for the presence of poly(A) in spinach chloroplast RNA by two methods; (1) hybridisation of chloroplast RNA with ³H-poly(U) and (2) binding of chloroplast RNA, pulse-labelled *in vivo*, to oligo(dT) cellulose. Additionally, the mRNA activities of chloroplast poly(A)-containing RNA and non-poly(A)-containing RNA (hereafter referred to as poly(A)⁺ RNA and poly(A)[−] RNA respectively) have been investigated by measuring their translation into specific polypeptides in cell-free extracts from *Escherichia coli*. From the results, we conclude that chloroplast mRNA lacks poly(A).

isolated from leaves labelled *in vivo* for 3 h with ³²P-ortho-phosphate¹⁴. For comparison of poly(A)⁺ RNA content, leaf total RNA was prepared from the same tissue. We have previously shown¹⁴ that in leaves pulse-labelled with ³²P-ortho-phosphate for 3 h, label is incorporated into mature rRNA, rRNA precursors and 'polydisperse' RNA in both chloroplast and leaf total RNA preparations. The poly(A)⁺ RNA content of the samples was determined by fractionation on columns of oligo(dT) cellulose as described by Clegg and Kennedy¹⁵. The results (Table 2) show that whereas 10.5% of the leaf total labelled RNA is retained by oligo(dT) cellulose, and is subsequently eluted in low salt buffer, only 2.1% of the RNA extracted from the crude chloroplast preparation is thus retained by oligo(dT) cellulose. A significant proportion of the poly(A)⁺ RNA extracted from the crude chloroplast preparation probably represents cytoplasmic contamination, since washing the chloroplasts once with isotonic medium before nucleic acid extraction reduces the poly(A)⁺ RNA content to about 1%; washing the chloroplasts a second time does not further reduce the poly(A)⁺ RNA content. Polyacrylamide gel electrophoresis of the ³²P-labelled poly(A)⁺ RNA fraction in leaf total RNA showed it to be heterogeneous in size, ranging from 0.2 × 10⁶ to 4 × 10⁶ molecular weight (not shown). In contrast, the poly(A)⁺ RNA from washed chloroplasts was much smaller in size, the majority migrating ahead of 4S RNA. This suggests that the small amount of poly(A)⁺ RNA in the washed chloroplast preparation does not simply arise from cytoplasmic contamination.

To establish whether or not it is the small amount of chloroplast poly(A)⁺ RNA, or the poly(A)[−] RNA which possesses mRNA activity, the two fractions were assayed for their ability to direct the synthesis of specific polypeptides in a cell-free protein synthesising system from *E. coli*. The products of cell-free protein synthesis were analysed by polyacrylamide gel

Table 1 Determination of poly(A) content by hybridisation with ³H-poly(U)

Source of RNA	Amount (μg)	RNase-resistant c.p.m.	c.p.m. minus no RNA control	Poly(U) in hybrid (μg)	% poly(A)
No RNA	—	87	—	—	—
<i>E. coli</i> ribosomes	50 100	78 83	—	—	ND ND
Bacteriophage MS2	50 100	350 608	263 521	0.0018 0.0036	0.0034 0.0034
Spinach leaf (total)	50 100	6 153 11,362	6,066 11,275	0.043 0.079	0.080 0.074
Spinach chloroplasts	50 100	330 621	243 534	0.0017 0.0037	0.0032 0.0035

Hybridisation mixtures contained the components of the standard assay described by Gillespie *et al.*¹³ 50 or 100 μg of the non-radioactive RNA shown and 1 μg of ³H-poly(U) (specific activity 143 × 10³ c.p.m. μg^{−1}; prepared as described by Eaton and Hutchinson¹⁶) in a total volume of 125 μl. After incubation at 36 °C for 24 h, 2.5 ml of 0.5 M NaCl, 10 mM MgCl₂, 10 mM Tris-HCl (pH 7.2) was added, followed by 15 μg RNase A and 5 μg DNase I, and incubation continued at 30 °C for 1 h. TCA-insoluble radioactivity was determined as described by Gillespie *et al.*¹³. The poly(A) content of the RNAs was calculated from the equation: poly(A) content = (μg hybrid poly(U) × 0.933)/(μg RNA) × 100. Spinach leaf total RNA, chloroplast RNA and bacteriophage MS2 RNA were prepared as described previously^{14,15}. *E. coli* rRNA was described by Avery¹⁷.

ND, Not detectable.

Samples of spinach chloroplast RNA were assayed for their poly(A) content by hybridisation with excess ³H-poly(U) in conditions reported to be optimal for poly(A)-poly(U) hybrid formation¹³. For comparison, samples of leaf total RNA (presumably containing poly(A) tracts), *E. coli* rRNA and bacteriophage MS2 RNA (the latter two lacking poly(A) tracts¹³) were similarly analysed. The results (Table 1) show that the poly(A) content of chloroplast RNA (0.003%) is some 25 times less than that of leaf total RNA, and is nearly identical to that of MS2 RNA (Table 1).

To investigate the poly(A)⁺ RNA content of pulse-labelled chloroplast RNA, RNA was extracted from chloroplasts

electrophoresis. The gel profiles obtained (Fig. 1) show that the product synthesised in the presence of chloroplast poly(A)[−] RNA (Fig. 1a) contains two discrete radioactive polypeptides of molecular weights 52,000 and 35,000. We have reported previously a very similar pattern of incorporation directed by unfractionated spinach chloroplast RNA, and established the identity of 52,000 molecular weight product as being the large subunit of Fraction I protein¹⁵. The identity of the 35,000 molecular weight product is at present unknown. The product synthesised in the presence of poly(A)⁺ chloroplast RNA (containing additionally 100 μg *E. coli* rRNA as carrier) contains no discrete, high molecular weight polypeptides

(Fig. 1b) and is qualitatively and quantitatively similar to the product synthesised in the presence of 100 μ g *E. coli* rRNA alone (Fig. 1c). From these results we conclude that the mRNAs for the large subunit of Fraction I protein and the 35,000 molecular weight polypeptide lack poly(A) tracts. This does not entirely exclude the possibility that other chloroplast mRNAs may contain poly(A), since they could be translated with a low efficiency in the *E. coli* cell-free system. This possibility is unlikely, however, since the products of incorporation directed by poly(A)⁻ RNA are qualitatively similar to those synthesised in intact, isolated chloroplasts¹⁵.

The most likely explanation of the results described here is that spinach chloroplast mRNA lacks poly(A); tracts of less than about 20 adenylate residues would not have been detected². In view of the complete lack of knowledge surrounding the

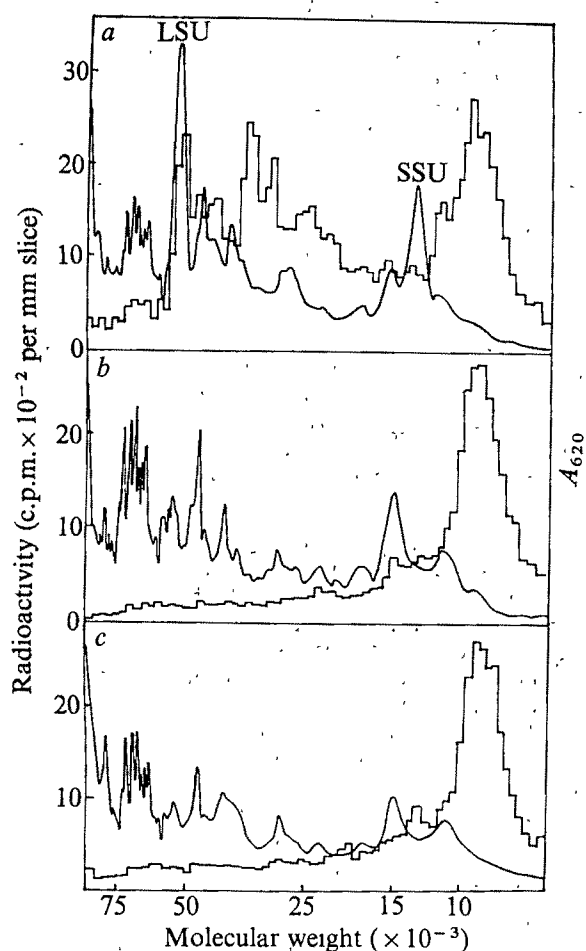


Fig. 1 Gel electrophoresis of the products of chloroplast poly(A)⁺ and poly(A)⁻ RNA-directed protein synthesis in cell-free extracts from *E. coli*. Chloroplast RNA was prepared as previously described¹⁴ and 0.5 mg aliquots separated into poly(A)⁺ and poly(A)⁻ RNA fractions on oligo(dT)-cellulose columns¹⁸. The poly(A)⁻ RNA, which passed through the column in 100 mM LiCl, 1 mM EDTA, 0.1% SDS, 50 mM Tris-HCl (pH 7.5) was ethanol precipitated directly from the eluant. The poly(A)⁺ RNA, eluted in low salt buffer (see legend of Table 2) was made 100 mM in NaCl; 100 μ g *E. coli* rRNA was then added as carrier before ethanol precipitation. A sample containing 100 μ g *E. coli* rRNA was similarly treated to serve as a control. These RNA samples were assayed for their ability to direct protein synthesis in *E. coli* S-30 extracts by methods previously described¹⁵. Products were analysed by SDS-polyacrylamide gel electrophoresis¹⁵. *a*, Incubation mix contained poly(A)⁻ RNA; *b*, incubation mix contained poly(A)⁺ RNA; *c*, incubation mix contained 100 μ g *E. coli* rRNA. —, Absorbance at 620 nm; histogram, radioactivity. LSU and SSU refer to the large and small subunits respectively, of Fraction I protein added to sample (*a*) as marker.

Table 2. Fractionation of pulse-labelled RNA on oligo(dT) cellulose columns

Source of ³² P-labelled RNA	Radioactivity applied to oligo(dT) cellulose column (c.p.m.)	Radioactivity eluted in low salt fraction (c.p.m.)	Poly(A) ⁺ RNA content (%)
Leaf (total)	50 × 10 ⁴	52,676	10.5
Crude chloroplasts	25 × 10 ⁴	5,251	2.10
Chloroplasts washed × 1	25 × 10 ⁴	2,623	1.05
Chloroplasts washed × 2	25 × 10 ⁴	2,717	1.09
<i>E. coli</i> ribosomes	100 × 10 ⁴	212	0.021

Leaves (5 g) excised from 15-d-old spinach plants were labelled for 3 h with ³²P-orthophosphate by way of their petioles¹⁴. Leaf total nucleic acid and crude chloroplasts were prepared¹⁴; for washed chloroplasts, the crude chloroplast pellet was resuspended in 5 ml of 0.35 M sucrose, 2 mM EDTA, 2 mM sodium isoascorbate and repelleted by centrifugation at 2,500g for 2 min. Chloroplast nucleic acid was prepared by the phenol-detergent method¹⁴. Ethanol-precipitated nucleic acid samples were dissolved at a concentration of 1 mg ml⁻¹ in 50 mM MES-NaOH buffer (pH 7.0), 2 mM MgCl₂ containing 5 μ g ml⁻¹ DNase I (RNase-free) and the samples incubated on ice for 20 min. Sodium dodecyl sulphate was then added to 0.5% and low molecular weight ³²P-labelled contaminants and DNase digestion products removed by exclusion chromatography on Sephadex G-25 columns. RNA samples were separated into poly(A)⁺ and poly(A)⁻ fractions on temperature-controlled oligo(dT)-cellulose columns as described by Clegg and Kennedy¹⁸. Poly(A)⁺ RNA was eluted from oligo(dT) cellulose with low salt buffer (10 mM Tris-HCl (pH 7.5), 0.1% SDS). The recovery of the radioactivity applied varied from 98% to 105%. ³²P-labelled *E. coli* rRNA was prepared as described by Avery¹⁷. Approximate specific activities of RNAs; leaf total, 4.1 × 10³ c.p.m. μ g⁻¹; chloroplast 2.3 × 10³ c.p.m. μ g⁻¹; *E. coli*, 34 × 10³ c.p.m. μ g⁻¹.

function of poly(A) in eukaryotic cytoplasmic mRNAs, no general significance can be placed on this finding at present, but it does reinforce the conclusion that poly(A) is not essential for messenger function^{19,20}. If the lack of poly(A) in chloroplast mRNAs proves to be a general phenomenon, it will be another respect in which chloroplasts resemble prokaryotes.

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Auxin, carbon dioxide and hydrogen ions

HYDROGEN ions can partially mimic the effect of indoleacetic acid (IAA) on plant cell elongation¹. This observation has been elaborated in a proposal that the initial action of IAA causes the activation of a proton pump at the cell membrane. The pumping of protons would cause a lowering of the pH at the cell wall, leading to wall loosening². This proposal has been supported in studies in which the pH of the medium surrounding oat coleoptile³⁻⁵ or etiolated pea stem⁶ sections was observed to drop when auxin was applied. This acidification was most pronounced when tissue sections were used from which the epidermis with the cuticle had been peeled off³, and was presumed to be caused by the proton pump activated by IAA.

An increase in cell respiration occurs when tissues respond to exogenous IAA (ref. 7). The IAA-induced pH drop observed in the solutions surrounding peeled tissue sections may be attributable to increased leakage of CO₂ from the tissue and subsequent formation of carbonic acid. To investigate this possibility, we measured CO₂ concentrations in the solutions surrounding pea epicotyl sections challenged with IAA.

Pea seeds (*Pisum sativum* L. cv. Alaska, lot 401 from Burpee Seed Co., Riverside, California) were germinated in the dark at room temperature (21 °C). Subapical 10-mm sections from the third internode were cut from 8-d-old seedlings. In peeled section experiments, the epidermis with cuticle was removed with fine forceps³. After the experimental period, the section lengths were measured, the pH of the medium taken, and the CO₂ contents of the solutions were measured with a Natelson model 600 microgasometer (Scientific Industries, Springfield, Massachusetts), which manometrically measures CO₂ in solutions.

Table 1 Effect of IAA on pH and CO₂ in the solution surrounding etiolated pea stem sections*

Conditions	pH ± s.d.	mM CO ₂ ± s.d.	Calculated pH
Unpeeled	6.04 ± 0.09	1.6 ± 0.09	6.02
Unpeeled + IAA	5.81 ± 0.10	3.8 ± 0.06	5.73
Peeled	5.87 ± 0.08	1.9 ± 0.09	5.97
Peeled + IAA	5.45 ± 0.11	5.3 ± 0.56	5.45
No sections	6.30 ± 0.00	0.5 ± 0.12	6.15

*Prepared sections were stirred in 200–300 ml 1.0 mM KH₂PO₄ buffer, pH 6.25 for 150–200 min. Rinsed sections were divided into lots of 45 and placed in 50 ml beakers containing 5 ml 1.0 mM KH₂PO₄ buffer, pH 6.30. After 30 min, the initial pH reading was taken and IAA was added to 10 µM. Beakers were sealed with Parafilm and allowed to stir for 3 h at which time readings were taken³.

Measurements of pH showed that IAA induced some acidification in the solution surrounding unpeeled sections, but that the acidification was more pronounced when peeled sections were used (Table 1). These data confirm previous results reported for unpeeled⁶ and peeled³ sections. The CO₂ measurements showed that when there was a drop in pH, there was also a rise in the CO₂ content of the solution. Using a *K_a* for total CO₂ in solution of 4.2 × 10⁻⁷ and a *pK_a* for the buffer used of 7.2 we were able to calculate the pH of the solution expected if only CO₂ was involved in the acidification⁸. These calculated pH values (Table 1) are quite similar to the measured pH, indicating that dissolved CO₂ was probably responsible for the IAA-induced pH reduction.

We carried out a time-course experiment to further examine the correlation between dissolved CO₂ and reduction in pH. When peeled sections were exposed for varying times to IAA, the results confirmed the correlation between increases in CO₂ and decreases in pH in the surrounding medium. Once again,

Table 2 Time course of pH and CO₂ changes in solution surrounding IAA-treated etiolated pea stem sections

Hours in IAA	pH	mM CO ₂	Calculated pH
0	6.1	0.5	6.15
1	5.8	3.4	5.73
2	5.6	4.4	5.63
3	5.3	5.8	5.43

the CO₂ effect was calculated to be adequate to account for the pH drop observed (Table 2).

To determine the relationship between the two correlated phenomena in solution, we carried out experiments in flasks with KOH-soaked filter paper in a centre well with the sections and solution surrounding the well. As the KOH would neutralise any CO₂-caused acidification, any pH reduction would then be caused by other phenomena. That little pH reduction was observed in such an experiment (Table 3) suggests a cause and effect relationship between increased CO₂ and reduction of pH during IAA treatment. It has been shown that exogenous CO₂ increases can induce cell elongation⁹. In all of our experiments, cell elongation (approximately 2 mm in 3 h) occurred only in IAA-treated tissue.

Table 3 Effect of KOH-soaked paper in a centre well on pH and CO₂ in solution surrounding etiolated pea stem sections*

Conditions	pH ± s.d.	mM CO ₂ ± s.d.	Calculated pH
Unpeeled	6.15	1.2	6.06
Unpeeled + IAA	6.11 ± 0.09	1.1 ± 0.08	6.07
Peeled	6.15	1.4	6.04
Peeled + IAA	6.09 ± 0.10	1.3 ± 0.09	6.04

*Sections were treated as described in Table 1, except that the experiment was run in a flask with a centre well, containing filter paper soaked with 10% KOH or water (control).

The source of the increased CO₂ in our experiments is probably increased cell respiration induced by IAA, the gas dissolving to form carbonic acid, which dissociates into bicarbonate and hydrogen ions. Plant cuticles are generally impervious to CO₂, and consequently the pH drop in the solution would be expected to be more pronounced in peeled than unpeeled section experiments¹⁰.

Our data tend to negate the hypothesis that the pH drop observed in the solutions surrounding IAA-treated tissue is attributable to a direct pumping of protons^{3,4}. It is possible that bicarbonate is a counter ion which accompanies hydrogen ion secretion. The data do not rule out the possibility that the primary event in cell elongation is indeed a lowering of the pH at the cell wall.

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matters arising

Interarc spreading in the Carpathian area

BOCCALETTI and GUAZZONE (ref. 1) have invoked the interarc spreading hypothesis to explain the genesis of the present structure of the Mediterranean area. We consider it necessary to clear up some fundamental points regarding one of the sectors they discussed.

The area inside the Carpathian Bend (including the Transylvania Basin in Rumania) was presented by Boccaletti and Guazzone as an interarc basin, the front and back inarc parts of which would be the Neogene volcanic zones in the East Carpathians and Apuseni Mountains. Because such a hypothesis has so far not been examined and argued but only stated²⁻⁴ we point out here the main geological facts which must be considered.

First, the basement of the Transylvanian Basin comprises both metamorphic and igneous (pre-Cenomanian) rocks, similar to those in the Carpathians⁵. Second, the sedimentary undeformed cover includes Palaeogene and early Miocene deposits continuous over large areas⁵. Third, there have so far been no observations indicating anomalous heat flow in this area.

The first point precludes any interpretation of the Transylvanian area as an interarc basin. If, however, this evidence were not considered, the second point would preclude a late Miocene age for the spreading process. Nevertheless, if the spreading were of that age, the Transylvanian Basin, being younger than the Pannonian Basin, should have a higher or comparable heat flow to that of the latter.

Finally, we emphasise that Karig⁶ developed the idea of interarc spreading for volcanic island arcs; his hypothesis cannot, however, be extended indiscriminately to apply to continental areas such as that inside the Carpathian Bend.

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DRS BOCCALETTI AND GUAZZONE REPLY

—The structural model comprising a consuming (or contracting) zone, a folded arc, a magmatic arc, and spreading (or distended) zones, after Karig's formulation¹ and after additional ideas by Dickinson² and Dewey *et al.*^{3,4} must be considered the best model to date for investigating fossil continental margins, both in wide, contracting palaeo-oceans and in small, contracting palaeomarginal basins. Differences between oceanic and continental arcs are probably only a matter of differences in the ages of the contracting processes and differences in the dimensions of contracting areas. Consequently, different stages of maturity probably also occur in areas beyond the arcs.

In that sense all Mediterranean basins, both present and fossils, are very different from those of the western Pacific. Even the Tyrrhenian Basin, which is generally accepted as a typical marginal basin in the Mediterranean area (with active subduction, typical polarity and migration, and typical folded and magmatic arcs) also shows a crustal substratum that is strongly thinned and very hot. It seems to be complicated, however, by many sialic microfragments and sialic seamounts. These sialic 'lenses' are probably actual examples of a segmentation stage in the pre-Tyrrhenian crust. The original floor of the Tyrrhenian Sea, as well as the substrata of the other Mediterranean basins, was never entirely substituted by typical oceanic crust.

The points raised by Radulescu and Sandulescu⁵ can be explained as effects of a low level of maturity of evolution in the Carpathian arc-trench system, where the back-arc crust has been distended, thinned and in places fragmented to a certain degree. In fact, Tortonian sediments directly overlie the basement in many places of the Transylvanian Basin⁶. Some spreading may occur under cover, caused by discontinuous subduction; perhaps that can be

correlated with rotated arc migration. Incidentally, the age of the oldest deposits contemporaneous with the spreading in the basin must be related to later surface volcanism.

Stegena⁷⁻⁸ suggest that the inter-Carpathian area should be considered to be "warm". Comparisons between the Pannonian and Transylvanian basins could not give the same result if the directions of the investigation profiles are changed. In fact, it has been clearly demonstrated that the northern Carpathians are inactive whereas the south-eastern Carpathian Arc is still active⁹. Geothermic correlations between different basins should take into account the different thicknesses of their covers. The only conjecture that we can advance about the comparison between the Pannonian and Transylvanian basins is that the second is probably less 'mature' than the first.

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LSD and dopamine receptors

PIERI *et al.*¹ reported a circling behaviour response to LSD in rats with unilateral chemical lesions of the ascending medial forebrain bundle. They used two rotational models; one was produced by 6-hydroxydopamine injection and the other by 5,6-dihydroxytryptamine injection. The factor common to both models was a deficit of dopamine in the ipsilateral forebrain. The authors demonstrated strong maximal contralateral turning responses to LSD given in a dose range of 0.1-1.5 mg kg⁻¹. Only the time course of turning seemed to be dose dependent.

We have repeated these experiments in similar animal models and have found the effects less convincing. Indeed, LSD

¹ Boccaletti, M., Guazzone, G., *Nature*, 252, 18 (1974).

produced apomorphine-like (contraversive) circling behaviour in only 40–50% of animals tested, and then at the very high drug levels of 1 and 1.5 mg kg⁻¹. In the turning mouse model of von Voigtlander and Moore², where 1 mg kg⁻¹ apomorphine induced a mean rate of turning of 5 min⁻¹, no satisfactory data for LSD could be obtained. In the dose range 0.025–0.2 mg kg⁻¹, LSD induced neither turning behaviour or postural asymmetries nor significantly modified apomorphine or amphetamine-induced circling, as may be predicted were it a potent dopamine agonist. Where rotation was induced following administration of 1.5 mg kg⁻¹ LSD the duration was of the order of 35–45 min; not 2 h as Pieri *et al.* suggest. We agree with them that rotation was prevented by previous treatment with haloperidol (0.5 mg kg⁻¹), and not after α -methyl-*p*-tyrosine (250 mg kg⁻¹).

In another experimental animal model, namely, audiogenic seizures in inbred strains of mice, dopamine agonists have been shown to diminish the severity of the seizure response^{3,4}. In our experiments, apomorphine was at least 10 times more potent than LSD in blocking the clonic phase of the seizure in 50% of animals.

The pharmacology of LSD is very confused, but has previously been associated with central 5-HT neurones^{5–7}. Pieri *et al.*, however, have claimed a direct and potent action of LSD on the dopaminergic receptor *in vivo*. Our data do not confirm LSD as being a potent dopamine agonist, although it seems to be capable of stimulating the dopamine receptor in very high doses.

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- ¹ Pieri, L., Pieri, M., and Haefely, W., *Nature*, **252**, 586–588 (1974).
- ² Von Voigtlander, P. F., and Moore, K. E., *Neuropharmacology*, **12**, 451–462 (1973).
- ³ Lehmann, A., in *Physiological Effects of Noise* (edit. by Welch, B. L., and Welch, A. S.), 227–257 (Plenum, New York, 1970).
- ⁴ Anlezark, G. M., and Meldrum, B. S., *Br. J. Pharmacol.*, **53**, 419–421 (1975).
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- ⁶ Chase, T. N., Breese, G. R., and Kopin, I. J., *Science*, **157**, 1461–1463 (1967).
- ⁷ Boakes, R. J., Bradley, P. B., Briggs, I., and Dray, A., *Br. J. Pharmacol.*, **40**, 202–218 (1970).

PIERI ET AL. REPLY—The results reported¹ correspond to a well defined model, namely rats unilaterally lesioned in the medial forebrain bundle with 5,6-HT or 6-OHDA. These two types of lesion have been extensively investigated with biochemical^{2,3} and histofluorescence methods (H. P. Lorez, unpublished), and result in a marked and long-lasting depletion of dopamine (DA), possibly leading to the subsequent development of denervation supersensitivity, (1 mg kg⁻¹

apomorphine being able to induce more than 15 turns min⁻¹ for 30 min). Lesioned animals were challenged with apomorphine and only those responding with a clear circling (about 50–60%) were subsequently used for the study of the effect of other agents, including LSD (80% of responses). The data obtained with several hundreds of rats have been described in part previously⁴ and a more extensive paper is in the press⁵. The fact that Pycock and Anlezark⁶ seem to have used a different animal species and a different lesion, clearly yielding less denervation supersensitivity (1 mg kg⁻¹ apomorphine inducing a mean rate of turning of 5 min⁻¹), precludes any reasonable comparison.

Concerning the relative potencies of apomorphine and LSD as striatal DA receptor agonists, it seems from our data that LSD is slightly more potent than apomorphine. By no means, however, do we claim that this applies to completely different models such as that reported by Pycock and Anlezark⁶ (namely, audiogenic seizures in mice).

It should also be stressed that low doses of LSD elicit a significant slowing of DA turnover in rat striatum (0.2 mg kg⁻¹ intraperitoneally) and retina (0.5 mg kg⁻¹ intraperitoneally). In addition, the striatal adenylate cyclase is activated by concentrations of LSD as low as 10⁻⁸ M (ref. 7). Thus, there is also biochemical evidence for DA receptor stimulating properties of LSD.

We did not intend to suggest that the DA receptor stimulant effect of LSD observed in our experimental conditions should be the final answer to the confused pharmacology of the compound. The DA-like component of LSD action in the central nervous system is, in our opinion, additive and not alternative to the stimulant action of the compound on 5-HT receptors. In circling behaviour, however, DA receptor stimulation seems to be important, at least in our model⁵.

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- ¹ Pieri, L., Pieri, M., and Haefely, W., *Nature*, **252**, 586 (1974).
- ² Saner, A., Pieri, L., Da Prada, M., and Pletscher, A., *Experientia*, **30**, 697 (1974).
- ³ Saner, A., Pieri, L., Moran, M., Da Prada, M., and Pletscher, A., *Brain Res.*, **76**, 109 (1974).
- ⁴ Pieri, L., and Pieri, M., *Experientia*, **30**, 696 (1974).
- ⁵ Pieri, M., Pieri, L., Saner, A., Da Prada, M., and Haefely, W., *Archs int Pharmacodyn.* (in the press).
- ⁶ Pycock, C., and Anlezark, G., *Nature*, **257**, 69–70 (1975).
- ⁷ Da Prada, M., Saner, A., Burkard, W. P., Bartholini, G., and Pletscher, A., *Brain Res.* (in the press).

Mimetic talking by parrots

THE paper by Gregory and Hopkins¹ has prompted me to speculate on the question; What is the biological sig-

nificance (or survival value) of 'talking' by parrots? Of course, parrots do not actually talk, they only imitate speech. In fact, they imitate, not only speech, but many sorts of discernible sound patterns that are likely to be produced repeatedly in their environment. Indeed, the articulated sounds, I suggest, signify a kind of 'functional' mimicry, whereby an animal is camouflaged by becoming one more source of its natural environmental noise. Yet, the animal does not presumably utter the mimetic sound patterns at random times and spaces, but rather it may utter them as responses to specific outer signals. This behaviour implies the existence of integrated neuronal circuits; and as a dominant role of the visual system is known in birds², the pupil constriction observed, suggests that certain (learned) visual patterns may act as signals (stimuli) that trigger talking behaviour. Thus, pupil constriction may serve to obtain clearer, optimal images of such visual patterns. Guzmán-Flores (personal communication) has found that parrots fail to learn recurrent, tape-recorded utterances, in which the correlative significant visual stimuli are missing.

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- ¹ Gregory, R., and Hopkins, P., *Nature*, **252**, 637–638 (1974).
- ² Polyak, S., *The Vertebrate Visual System* (University of Chicago Press, 1957).

Hereditary persistence of foetal haemoglobin

ALTHOUGH the interpretation of Martinez and Colombo¹ may be correct, their conclusions are based on uncertain evidence and an alternative explanation that fits within the classical scheme is equally plausible. Their argument rests entirely on the statement that "foetal haemoglobin (HbF) levels show intrafamilial segregation in β^{thal} ". From this statement, they conclude that III₂ cannot simply be a β^{thal} heterozygote like I₂. Our experience shows that this is not invariably so: parents, offspring, and siblings who are β^{thal} heterozygotes may differ in level of HbF. Consequently, we believe that III₂ is only a β^{thal} heterozygote and that his slightly elevated HbF is the result of his particular expression of the condition. The inheritance pattern then follows normally. I₁, II₁, and III₁ all have a chromosome with S and hereditary persistence of foetal Hb (HPFH); II₁ also has β^{thal} ; III₁ and III₂ have a normal chromosome from II₂.

The presence of HbS and HPFH in the *cis* form has not been reported, but is entirely feasible from our knowledge of the genetics and arrangement of the complex of β , γ , and δ genes². A type of HPFH which has only about 5% HbF has been described³. We have recently examined a family in which, in contrast to other HPFH classes, β^A chains are produced in *cis* to HPFH (ref. 4) and have speculated that a type of HPFH with production of β^S chains in *cis* is possible. Martinez and Colombo may have detected this combination. They report, however, a heterogeneous intracellular distribution of HbF in I_1 and III_1 , whereas a homogeneous distribution is one of the criteria of HPFH. Consequently, it is more probable that I_1 and III_1 merely have sickle cell trait with somewhat elevated HbF.

In summary, we believe that Martinez and Colombo have misjudged the genetic aspects of several members of this family and have made unwarranted interpretations. In all probability, I_1 and III_1 have sickle cell trait, I_2 and III_2 have β^{thal} trait, and II_1 has S- β^0 -thal.

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¹ Martinez, G., and Colombo, B., *Nature*, **252**, 735 (1974).

² Huisman, T. H. J., et al., *Ann. N.Y. Acad. Sci.*, **232**, 107 (1974).

³ Sukumaran, P. K., et al., *Br. J. Haemat.*, **23**, 403 (1972).

⁴ Huisman, T. H. J., Miller, A., and Schroeder, W. A., *Am. J. Human Genet.* (in the press).

DRS MARTINEZ AND COLOMBO REPLY—The argument put forward by Schroeder and Huisman¹ in their criticism to our paper² rests entirely on the sentence: "it is more probable that I_1 and III_1 merely have sickle cell trait with somewhat elevated HbF (foetal haemoglobin)", as confirmed by the last sentence of their letter. With this statement they want to imply that the elevation of HbF is determined by some non-genetical factor(s). This means that to interpret the elevated percentages of HbF in I_1 , III_1 and III_2 the following assumptions must be made. A subject and his grandmother, both showing HbS trait only, have exceptionally elevated percentages of HbF which are exactly the same (and by definition are not genetically determined); and a simple β^{thal} carrier is doubly exceptional because this subject not only shows HbF levels exceptionally high for this condition³, but also these levels do not show the intrafamilial segregation

reported by many authors (see, for example, refs 3 and 4). We consider that it must be very exceptional as only the opposite situation has been reported in the literature.

Being somewhat reluctant to postulate so many unwarranted hypotheses, we preferred to propose just one, that is, that in our family there was a segregation of a type of hereditary persistence of foetal haemoglobin (HPFH).

If we are in the presence of a type of HPFH the hypothesis that this HPFH is in *cis* to β^S (as mentioned and rejected also by Schroeder and Huisman) cannot be accepted for the following reasons: the ratio β^S/β^A was normal in I_1 and III_1 ; the gamete transmitted from II_1 to III_2 should be a recombinant between HPFH and β^S .

Thus the fact that the gene for this HPFH could not be in *cis* to β^S nor to β^A (see pedigree) enabled us to claim that we were in the presence of a "new type of HPFH".

It is evident then that the objection raised by Schroeder and Huisman concerning the intracellular distribution of HbF becomes completely irrelevant.

In summary, we believe that Schroeder and Huisman have misjudged the genotypes of all the members of this family showing elevated percentages of HbF. In fact trying to reject the most logical interpretation of our findings, they were forced to discard *tout-court* the possibility that this persistence of HbF was hereditary, thus creating for each member of the family the unwarranted, although unexpressed, number of necessary hypotheses.

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¹ Schroeder, W. A., and Huisman, T. H. J., *Nature*, **257**, 70-71 (1975).

² Martinez, G., and Colombo, B., *Nature*, **252**, 735 (1974).

³ Weatherhall, D. J., and Clegg, J. B., *The thalassaemia Syndromes* (Blackwell, Oxford, 1972).

⁴ Friedman, S., Hamilton, R. W., and Schwartz, E., *J. clin. Invest.*, **52**, 1453 (1973).

Ultraviolet light and human cataract

WEITER and Finch¹ could find no difference in paramagnetic species between normal and cataractous human lenses but demonstrated that prolonged ultraviolet irradiation of normal human lens produced free radical species, which they presumed were derived from tryptophan. They suggest that the production of free radicals by ultraviolet light might be a mechanism for photoinduced lens damage.

The theory that the ultraviolet radiation of sunlight (or from other sources) can cause brown nuclear cataract has been the subject of prolonged discussion, but there are two major arguments against such a

theory²: (1) The proteins of the brown cataractous nucleus do not show a loss of tryptophan compared with the normal human lens³, whereas a substantial loss of tryptophan is found in the products of *in vitro* photo-oxidation of lens proteins³; and, (2) in brown nuclear cataract, only the lens nucleus is pigmented and it is difficult to see how ultraviolet light could act on proteins of the lens nucleus alone, rather than those of the cortex, which are very similar; especially as absorption of ultraviolet by the cornea and outer layers of the lens would ensure that little harmful radiation could reach the centre of the lens.

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¹ Weiter, J. J., and Finch, E. D., *Nature*, **254**, 536-537 (1975).

² Dilley, K. J., and Pirie, A., *Expt Eye Res.*, **19**, 59-72 (1974).

³ Buckingham, R. H., and Pirie, A., *Expt Eye Res.*, **14**, 297-299 (1972).

WEITER AND FINCH REPLY—The invariance of tryptophan content in normal and cataractous lenses as opposed to the loss of tryptophan in the *in vitro* photo-oxidation of lens proteins may result from the likelihood that the tryptophan in the lens proteins *in vivo* may absorb the radiation and transmit the energy to some other species (for example, lipids) to produce the radical, which may in turn cause damage to the lens. If this is so, the tryptophan would remain essentially unaffected. Steen¹ has shown that tryptophan in an ethylene glycol-water glass at 77 K absorbs ultraviolet light to produce the radical in the solvent, and tryptophan itself may not form the radical. The growth of radical, however, in both Steen's and our own (unpublished) experiments with lens material is not linear with respect to exposure time, thereby indicating the possibility of some degradation of tryptophan and its consequent unavailability for excitation. It is likely that this could be the result of a mechanistic detail rather than tryptophan degradation. Production of a radical through tryptophan excitation may involve several steps and this may diminish the efficiency of radical production.

The second point we would make is that Steen¹ has pointed out that the formation of free radicals by ultraviolet light on tryptophan in ethylene glycol-water glass is accompanied by significant coloration of the sample and this was attributed to trapped electrons. The trapping was most efficient in the presence of a substantial concentration of H^+ ions, which are known to scavenge electrons very well. If we assume that the cataract coloration results from trapped electrons, a differential cationic concentration between the cortex and the nucleus could explain this nuclear pigmentation through a colour

centre formation. Alternatively, the coloration may arise from the formation of stable lipid peroxides, although in this case, we do not know why the nucleus is favoured discriminately for the production of lipid peroxide radicals.

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¹ Steen, H. B., *Photochem. Photobiol.*, 9, 479 (1969).

Reversibility and biological machines

GRAY¹, in discussing reversibility and biological machines, applied a formula derived by Brillouin², describing the energy required to determine the positional limits of a microscopic system, to the muscle cross bridge. A result of his analysis is that the cross bridge is too small to be controlled directly by any internal system which matches the tension to the external load, and that control must extend over greater distances; I doubt if anyone would contest this. Gray then, however, extends the concept of 'profitable controllability' from this restricted example to all machines of molecular dimensions which operate cyclically and argues that their mode of operation involves an irreversible, unidirectional step; if this is not so, then the efficiency of energy conversion must be very low indeed. This idea is identical to that of McClare³, who has argued that muscle contraction is an irreversible quantum-mechanical process whereby the free energy from ATP is converted to mechanical work. In fact, perfectly workable, self consistent models of muscular contraction can be made without any recourse to quantum mechanics, by general methods applicable to any energy conversion process⁴, but Gray claims to advance a definite proof that this is impossible and that biological molecular machinery cannot be considered as an energy converter in the classical thermodynamic sense.

This does not follow, however, from Gray's idea of 'profitable controllability' which only has meaning when the rate of working of a molecular machine is geared to the prevailing force-field (thermodynamic gradients or whatever) by some cybernetic process, to achieve a thermodynamically reversible operation. If this criterion does not apply the 'controllability' of the system is meaningless, or rather irrelevant. There is no evidence that molecular machines must be controlled in this way, and so Gray has only produced a proof for one highly restricted set of conditions. It is perhaps worth considering the case of such molecular machines

as ion pumps in the cell membrane, which can be driven backwards and which seem to function with high efficiency⁵. It is not possible to argue that there is no direct energy conversion occurring (as in a system involving mechanical work) because a substantial part of the work performed is that of moving a charge through an electric field gradient; that is, free energy from ATP is being used directly, by way of a conformational change, to achieve translation in a force-field. This must be taken to be a direct experimental disproof of Gray's thesis.

In summary, although the rate of energy conversion by a biological machine of molecular dimensions is subject to microscopic variation within the statistical limits imposed by the Second Law, there is no proof that size alone has any direct connection with the overall efficiency of the energy conversion process, or its potential reversibility.

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¹ Gray, B. F., *Nature*, 253, 436 (1975).

² Brillouin, L., *Science and Information Theory* (Academic, New York, 1962).

³ McClare, C. W. F., *Nature*, 240, 88 (1972).

⁴ Hill, T. L., *Prog. Biophys. molec. Biol.*, 28 (1975).

⁵ Garraban, P. J., and Glynn, I. M., *J. Physiol., Lond.*, 192, 217 (1967).

GRAY REPLIES—In the first few lines of his comment Hill¹ incorrectly paraphrases the contents of my letter, then identifies his version with a theory of McClare² (to which I did not even refer) which has been published for some time. He attributes to me claims which I do not make, for example, to advance a definite proof that "perfectly workable self-consistent models of muscular contraction cannot be made without recourse to quantum mechanics". Perfect workability, however, depends on how much or how little detail one is satisfied with in a theory. Hydrogen and oxygen will react explosively to form water

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

and the statement $\Delta G < 0$ and its implications may be a 'perfectly workable self-consistent model' to some people who are interested only in distinguishing potential explosives from inert material. To the man interested in why the reaction is explosive, at one temperature and almost immeasurably slow at a temperature one degree lower, however, this 'perfectly workable' model is completely useless! He needs the macroscopic theory of chain reactions, and a third man with an even greater sense of curiosity needs quantum mechanics to understand the magnitudes of the reaction cross sections involved in this theory.

The first part of Hill's second paragraph reveals a misunderstanding of my main thesis, which is that very small (molecular) machines cannot be individually controlled if they are to work efficiently, not the reverse. He argues that there is no evidence that biological molecular machines are controlled "by gearing to some prevailing force field to achieve a thermodynamically reversible operation". He is here reiterating the view expressed by McClare³, and my letter shows this is necessarily true, from a consideration of quantum theory and the observed sizes and efficiencies of molecular machines. We seem to agree that the independent working units, such as a crossbridge or a pump site, cycle autonomously in normal conditions in their forward direction, that is, using ATP. Clearly they cannot be made to go in reverse without the intervention of control, by definition, thus giving a concomitant drop in efficiency for units of this size. Experimental results on the reversal of ion pumps³ do not conflict with this conclusion, since the reversal of the ion pump obtained in human red cells was achieved in highly abnormal conditions, not the normal environment of these cells and the energy expended in producing this abnormal environment must not be ignored in any discussion of efficiency and reversibility. Obviously any cyclic machine can be run backwards if one goes to sufficient lengths to push it, but there will be a drastic loss in efficiency except in the classical limit, where one can operate reversibly without energy cost. My brief statement about the reversal of muscle should be understood in this sense.

Hill's last statement does not seem to make sense, as the Second Law imposes no limits, statistical or otherwise, on the rate of energy conversion of any machines from molecular biological to macroscopic steam engines. It gives a criterion for distinguishing processes in which work can potentially be extracted from others in which it cannot.

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¹ Hill, A. E., *Nature*, 257, 72 (1975).

² McClare, C. W. F., *J. theor. Biol.*, 30, 20 (1971).

³ Glynn, I. M., Lew, V. L., and Luithi, U., *J. Physiol., Lond.*, 207, 371 (1970).

reviews

RIBOSOMES are subcellular organelles that are found mainly in the cytoplasm. They are important because they are the site of protein biosynthesis, where the linear nucleotide sequence of messenger RNA is translated into the linear amino acid sequence of a particular protein. They may be regarded as multifunctional multisubunit enzymes and they are unusual because they contain RNA as a major component.

Ribosomes were first isolated and studied about 25 years ago. James Watson and Alfred Tissières began the study of the bacterial ribosome in the late 1950s and the complexity of the particle (at least 3 RNA species and at least 55 different protein species) began to emerge. For a time, during the period when the amino acid code was being solved, it was convenient to regard the ribosome as a black box. Around 1968 several developments took place that allowed a study of the black box itself. These developments included: the isolation of individual ribosomal proteins; the reassembly of functional subribosomal particles from their RNA and protein components; the preparation of antibodies to individual proteins and the invention of other techniques for analysing complicated protein mixtures; the emergence of nucleotide sequencing techniques; and the choice throughout the world of *Escherichia coli* ribosomes as the preferred system for study—this concentration on a single species has proved specially important—not least in promoting collaborative research.

This book arises from a meeting on ribosomes held in Cold Spring Harbor in September 1973 and fixes the view of the bacterial ribosome (particularly the *E. coli* ribosome) as it appeared at the meeting. The book has many admirable

Ribosomes

R. A. Cox

Ribosomes. By M. Nomura, A. Tissières and P. Lengyel. Pp. x+930. (Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 1974.) \$32.00.

features. The first part comprises a series of general reviews. The editors have carefully chosen the contributors from those invited to the meeting, and a few who were themselves the pioneers responsible for some of the most recent advances were asked to provide more general surveys of topics such as RNA-protein interactions in the ribosome, the protein topography of ribosomal subunits, and the reconstitution of ribosomes, as well as reviews of physical properties of ribosomes, and of the isolation and properties of RNA and proteins. The process of translation, ribosome genetics and ribosome biosynthesis are also reviewed. The reviewers generally produced more than a factual survey and have emphasised where possible the relationship between structure and function. By no means all of the progress achieved in ribosome research has a chemical basis but a major impression remains that a chemical approach has proved to be fruitful for probing ribosome structure and eroding the 'black box' concept. For example, it can now be said "that the fidelity with which messenger RNA can be translated depends critically on the amino acid residues at positions 42 and

87 of protein S12". Bifunctional cross-linking agents have proved useful in identifying those proteins that are neighbours in the ribosome, and affinity labelling methods have been used increasingly to identify functionally active groups.

The second part of the book is made up of a series of carefully selected "Specific Reviews" of topics such as electron microscopy, affinity labelling techniques, immunochemical analysis, fluorescence studies, ribosome genetics, "magic spot", RNA synthesis *in vivo* and *in vitro* and particular aspects of ribosome function. There are three specialist reports on antibiotics and the ribosome but no general review of this topic although widespread use is made of these substances as probes of ribosome structure.

The glory belongs to the *E. coli* ribosome but about a fifth of the book is devoted to ribosomes of eukaryotes. There is one chapter and one specific report devoted to eukaryotic ribosome structure. This emphasis is, however, a fair reflection of the field as a whole.

The book comprises 930 pages and a comprehensive index. It provides a very clear picture of the *E. coli* ribosome as it was seen in late 1973 with the proteins preeminent and the RNA moiety in the shadows; and it also reveals and documents the remarkable progress made over a five-year period. It is a monumental effort that does justice to the subject—indeed, it is the most ambitious publication in the field so far—and the standard of precision and clarity it achieves in giving shape to so many diverse facts is very high. This book has much to offer those who are interested in nucleoproteins and it is compelling reading for those who are actively interested in ribosomes.

THE concept of aromaticity in organic molecules, which has done so much to inspire experimental work in both mechanistic and preparative areas, is still the subject of much debate and controversy. The present volume sets out to present the varied aspects of aromaticity in a critical and concise manner and the result is a very readable account of the present status of the subject. What is lost in detail in the text is compensated for by an extensive list of references so that the reader will have no difficulty in

Aromaticity

A. W. Johnson

Facts and Theories of Aromaticity. By David Lewis and David Peters. Pp. viii+109. (Macmillan: London and Basingstoke, April 1975.) £10.00.

following up any particular aspect. After a brief historical introduction, chapters are given on experimental evidence,

theoretical ideas and simple examples of aromaticity and a variety of non-benzenoid structures: for example, quinoids, pyrones and porphyrins are also considered. Finally, ideas on homoaromaticity and antiaromaticity are summarised and some general conclusions are advanced. It is an interesting text on a topic of interest to all organic chemists and it is well written and produced in an attractive format, as indeed would be expected for about 10 pence a page.

Books for Autumn 1975

Fish Communities in Tropical Freshwaters

R H Lowe-McConnell

By reference to ecological studies in freshwater fishes, this book illustrates how tropical fish communities differ from those in temperate waters. The book makes available to the general ecologist the results of recent research into the ecology of the fish.

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F D Gunstone

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D W Young

An introduction to the chemistry of heterocyclic compounds for students of chemistry, pharmacy and biochemistry, who possess a basic knowledge of biochemistry. The reactions of heterocyclic compounds are compared with those of their simple organic-functional group analogues by noting how ring and heteroatoms interact to produce the unique properties exhibited by heterocyclic compounds.

The chemical effects of aliphatic heterocyclic compounds are discussed, and consideration is given to aromaticity and tautomerism, leading to a discussion of hetero-aromatic compounds in general.

Probably £3.50 net



Longman

Quantum optics

Introduction to Quantum Optics. (Documents on Modern Physics.) By H. M. Nussenzveig. Pp. xiv + 246. (Gordon and Breach: London, New York and Paris, December 1974.) DM 59; \$24.40.

Optical Resonance and Two-Level Atoms. (Interscience Monographs and Texts in Physics and Astronomy, vol. 28.) By L. Allen and J. H. Eberly. (Wiley Interscience: New York and London, March 1975.) £10.80.

ALTHOUGH the range of topics discussed in these books is not identical there is, nevertheless, some relationship between them. One obvious link is that Professors Nussenzveig and Eberly are both in the same department of the University of Rochester. Furthermore, the books are of comparable length and depth, and are clearly intended for the same type of audience. And both include chapters on superradiance, a topic that has so far received little text book coverage.

In *Introduction to Quantum Optics*, the foundations of the subject are treated in a fairly standard way. The bulk of the book is based on a series of lectures presented by the author at the Latin American School of Physics in La Plata in 1970, and is devoted to the classical and quantum theories of optical coherence and the semiclassical and quantum theories of the laser. A chapter has since been added on coherent interactions, in which superradiance, photon echoes and self-induced transparency are described from a semiclassical viewpoint. The book closes with a rather disjointed 30 page appendix on recent developments (much of which is devoted to the quantum theory of superradiance), which certainly serves to emphasise the unfinished nature of quantum optics research. The style throughout is very readable and there are adequate references at the end of each chapter.

The book is by no means unique in its coverage, apart from perhaps the welcome inclusion of superradiance. In his introduction, Professor Nussenzveig states that the book is "aimed at theoretical physicists and graduate students . . . as an introduction to a new subject with which no previous familiarity is assumed". One happy consequence is that the level of presentation is not so high that the experimentalist will immediately be daunted. In this, perhaps, lies the book's particular merit.

Optical Resonance and Two-Level Atoms is more original in conception, bringing together material that has until now been widely scattered in other books or reported only in research journals. Indeed, it forms an excellent sequel to *Introduction to Quantum Optics*, being devoted entirely to the resonance phenomena treated in the last chapters

of Professor Nussenzveig's book. Both authors have published extensively in this field although the stated intention of the book is not to emphasise recent research but rather to reveal the general principles involved. The first chapter deals with the classical theory of absorption from an unconventional point of view which allows the authors to introduce the idea of pulse "area". An examination of the validity of the two-level assumption and a useful discussion of the various other approximations commonly used leads on to an extended treatment of Bloch vector dynamics and self-induced transparency, which forms the central section of the book. Experimental results are compared with the theoretical predictions, the possible confusion of self-induced transparency with incoherent saturation effects being carefully explained. A chapter on single-atom spontaneous emission using quantised field theory lays the groundwork for the discussion of cooperative phenomena, superradiant decay and photon echoes which forms the second part of the book. The style of writing is clear and informal and the emphasis throughout is always on the physics of the processes taking place. There are numerous helpful illustrations and adequate lists of references.

Both *Introduction to Quantum Optics* and *Optical Resonance and Two-Level Atoms* represent welcome additions to the literature on quantum optics. The latter will prove particularly valuable because of its unusual coverage, although both can be warmly recommended.

G. H. C. New



Pictish horse and foot soldiers form a battle scene on the back of a Pictish sandstone cross slab, Aberlemno churchyard, Aberlemno, Angus, Scotland. Taken from *Scotland: An Archeological Guide* by Euan W. Mackie. Pp. x + 298. (Faber and Faber: London, June 1975.) Cloth £5.50; paper £2.95.

Seismic Waves. By E. F. Savarenskii. Translated from Russian. Pp. vi+281. (Israel Program for Scientific Translations: Jerusalem; Wiley: Chichester; May 1975.) £13.95.

THIS book, excellently translated, caters mainly for the physicist who wants to study elastodynamics and that part of data-handling needed with seismic data. There are three chapters: the first, on oscillations, deals mainly with Fourier analysis and operations in the frequency domain; the second, on waves, derives the basic equations of elastic wave motion and goes on to obtain radiation fields for point sources in an unbounded medium; the third describes the propagation of plane seismic waves in a half-space or a layer.

The book is written at a fairly elementary level, and mathematical demonstrations are sketchy. It omits, for instance, mention of the splitting of degenerate eigenfrequencies of the Earth, or of matrix methods for layered media. The reader will not find such recent topics as earthquake prediction, lunar seismology or deep seismic sounding. Within its scope, Savarenskii's book is clear and practical; it has obviously grown out of the experience of an expert seismologist. **E. R. Lapwood**

Bee Research Association, 1949-1974: A History of the First 25 Years. Pp. 199. (Bee Research Association: Gerrards Cross, 1974.) np.

LAST year was the 25th anniversary of the founding of the Bee Research Association and to help commemorate the first quarter of a century a history of the association, to which many of its prominent members have contributed, has now been published.

The history traces the foundation of the Bee Research Association as an offshoot of the research committee of the British Beekeepers' Association, its growth in size and importance to an international organisation and its acquisition of a permanent headquarters at Hill House in Chalfont St Peter. During this process it took over publication of the well established journal *Bee World*, introduced *Apicultural Abstracts*, first as an integral part of *Bee World* in 1950 and then as a separate quarterly journal in 1962, and launched *Journal of Apicultural Research* in 1961 in response to a strongly felt need for a journal devoted to experimental work relating to pollination and beekeeping.

Bee research workers and advisory officers readily acknowledge the value to their work of these three journals and the many other facilities the association provides, including its library of re-

prints, books and translations, all of which are discussed at length in this book. With the recent increase in beekeeping and in the use of bees for pollination, especially in developing countries, the services of the association are in greater demand than ever before. It is vital that they should continue to grow.

John B. Free

Books brief

Atomic Physics.. (The Manchester Physics Series.) By J. C. Willmott. Pp. xiv+357. (Wiley: London and New York, April 1975.) £8.50 cloth; £4.25 paper.

THIS textbook is intended for the second year of the honours physics course at an English University, specifically, Manchester, whose reputation in atomic and molecular physics is high. The book contains the necessary quantum mechanics, and inevitably, material that might be taught in the first year, and material that would in the opinion of some belong in the final year. But it stops short of Lamb shift and hyperfine structure. The author is primarily a nuclear physicist, and it is to some extent a reflection on atomic physicists that the subject is not particularly well served by undergraduate texts; for this reason, the book will fulfil a useful role. It is a competent book, although not everyone will find it inspiring. There are sets of problems in each chapter, and it is well produced and indexed.

J. B. Hasted

Registry of Mass Spectral Data; volumes 1-4. Edited by E. Stenhagen, S. Abrahamsson and F. W. McLafferty. Pp. xvii+3,358 (Wiley, New York, 1974.) £250.00.

WHEN it was first developed and became commercially available in the early 1960s, mass spectrometry was hailed as the panacea that would take the pain out of structure elucidation. No longer would organic chemists have to slave for months, if not years, degrading and analysing new compounds; instead a mass spectrum would tell all, and even a milligram of material would be sufficient. But this early euphoria was not wholly justified, and since then in the years of appraisal that followed, the limitations and the complications of mass spectrometry have become apparent. Although the expert practitioner, backed up by expensive and elaborate computing facilities, is able to

combine mass spectral fragments and deduce the structure of his unknown, the average organic chemist can deploy neither expertise nor facilities on this scale. Instead, he merely compares the spectrum of his unknown with those of known compounds and attempts to spot significant similarities.

This is where the *Registry of Mass Spectral Data* fits in. It is an immense and invaluable collection of reproductions of 18,806 authentic mass spectra. The spectra themselves are easy to locate, being arranged in order of increasing molecular weight (to the nearest whole mass unit); there is also an excellent index. Although expensive, the cost is, in fact, less than two pence a spectrum, and when the price of even the most modest mass spectrometer is considered, the additional expense in purchasing this unique compendium of spectra as an accessory is easily justified. I strongly recommend the *Registry of Mass Spectral Data* as a must for every laboratory in which mass spectrometry goes on; it cannot afford to be without one.

E. J. Thomas

Biological Transport. Second Edition. By Halvor N. Christensen. Pp. xvi+514. (Benjamin: Reading, Massachusetts and London, April 1975.) \$19.50.

NOWADAYS, the term 'Biological Transport' concerns the nature of, and mechanisms underlying, the transport of substances between different phases separated by membranes (for example, plasma and mitochondrial) or by epithelial structures (for example, intestine, placenta, renal tubule).

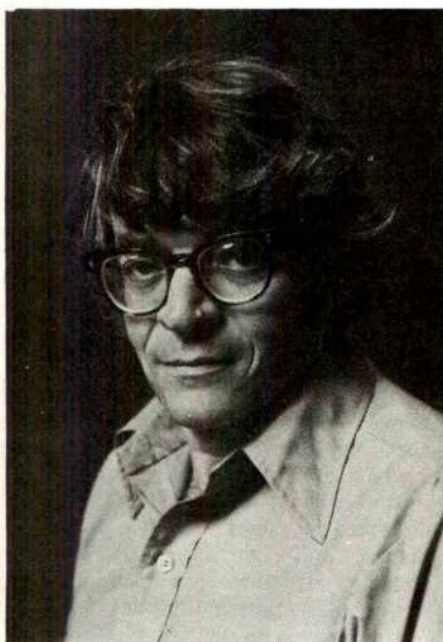
This second edition of H. N. Christensen's excellent book is really a complete new work and the author is to be congratulated on attempting to embrace within one volume all aspects of epithelial transport and membrane structure and function, including kinetic, thermodynamic and genetic aspects of real and artificial systems. He has largely succeeded in surmounting the disciplinary prejudices that seem to have caused an exceptional degree of fragmentation in the subject, and the book will accordingly be useful to workers studying membranes and epithelia.

The account of the ATPases seems somewhat old fashioned; vitamin B₁₂ is not absorbed in the duodenum; the electron micrograph of the erythrocyte is an antique; and after page 367 the contents page and the text become out of phase. But there is much in this book for those interested in any aspect of biological transport, and that must include biochemists, morphologists, physiologists and molecular biologists.

Dennis S. Parsons

obituary

Gordon Mayer Tomkins of the University of California, San Francisco, one of America's most distinguished biochemists and a pioneer research scientist in the field of hormone activity, died in New York City, July 22, 1975. He was 49 years old. He had been ill since undergoing brain surgery in late May.



To his colleagues in scientific institutions all over the world Dr Tomkins was an extraordinary individual. He was a physician, a highly creative scientist, a teacher of unparalleled enthusiasm and an accomplished musician who played both classics and jazz with distinction.

Dr Tomkins was born in Chicago and received an A.B. degree in Philosophy from UCLA, an M.D. degree from Harvard Medical School and a Ph.D. degree from the University of California, Berkeley. He spent 16 years at the NIH, Bethesda, Maryland, and left there in 1969 as Chief of the Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, to join the University of California, San Francisco, as Professor of Biochemistry.

He served on numerous editorial boards for scientific journals and

periodicals and was the recipient of many honours, including the Mider Lectureship (NIH, 1969); the Jesup Lectureship (Columbia, 1971); the Harvey Society Lectureship (Rockefeller, 1972); the Prather Lectureship (Harvard, 1972); and the Baker Lecture (Cornell, 1975).

His scientific contributions encompass broad aspects of biological chemistry and molecular biology. His first publications described the regulation of cholesterol biosynthesis by diet. From this early involvement with sterols developed his intense interest in steroid hormones and their molecular mechanisms of action. He described the now important enzymatic reductions and hydroxylations of steroids and soon after introduced the concept of and provided evidence for a low molecular weight compound, such as a steroid hormone, specifically changing the conformation of a protein molecule. This concept, subsequently termed allostery by others, is an important one not only in endocrinology but for most of molecular biology and biochemistry.

He was responsible for developing a system in which the mechanism of steroid action and the regulation of gene expression can be studied in continuously cultured mammalian cells, a system which is widely used in research laboratories today and which in recent years he extended to the selection and isolation of mutant cell clones with altered responses to steroid hormones and cyclic nucleotides. This approach is providing insights to many of the fundamental problems in endocrinology and is playing a significant role in the further acquisition of knowledge in fields of genetics, cellular and developmental biology, and cancer.

Equally important were his theoretical contributions to a variety of biological and medical fields. He introduced the idea that the regulation of the levels of specific proteins might occur at steps subsequent to the formation of their messenger RNA molecules. From a consideration of the problem of the transformation of a normal cell into a malignant or cancerous one with uncontrolled growth, he developed a unifying theory of growth regulation termed 'pleiotypy' by which a normal cell, unlike its malignant counterpart, coordinates a diverse set of intracellular reactions to arrest its growth in response to extracellular

conditions. In his final theoretical contribution, he described a plausible mechanism to account for the evolution of hormonal regulation, that is characteristic of multicellular organisms, from simple unicellular organisms.

His impact on medical research has been generated not only by his training and education of numerous esteemed scientists and physicians throughout the world but also by the unusual excitement about science that he created among colleagues and acquaintances.

To those who knew him scientifically, his death is the loss of exciting ideas; to those who knew him musically, the loss of distinguished talent; to those who knew him personally, the loss of intellectual brilliance, extraordinary wit, great warmth and charm. To those who were fortunate enough to experience all of these various gifts, his death is the end of an era.

Reiji Okazaki, the Japanese molecular biologist, died on August 1 at the age of 44.

Okazaki arrived at the forefront of molecular biology in 1966 with his discovery that the synthesis of new DNA strands during the process of DNA duplication seems to occur in rather short sections (now known to everyone as 'Okazaki pieces') that only later are joined together to make the long uninterrupted strands of the finished product. This finding explained how two new strands of opposite polarity could be synthesised at a single locus travelling in one direction along the parental double helix, and it therefore reconciled the properties of DNA polymerase with the large-scale structure of replicating DNA. Since then he had worked indefatigably to determine what is the direction of synthesis of the short pieces of DNA, how they are started and how finally they are joined together. These experiments involved a very high level of technical sophistication and were followed with great interest by all workers in the field. Okazaki was a schoolboy in Hiroshima when the bomb was dropped and this may have been the cause of his death, from leukaemia—a young man still at the height of his powers. At the time of his death he was professor of molecular biology at Nagoya University in Japan.

nature

September 11, 1975

Don't rush them to predict earthquakes

MORE people died in Turkey last Saturday as a result of an earthquake than have ever died in the United States from earthquakes. And yet earthquakes in the United States attract an enormous global attention. One reason is simply human curiosity that a technological leader can be laid low by acts of God; for much the same reason are medical bulletins of heads of state read with fascination. A more subtle reason is the extraordinary way in which the lessons of every new major earthquake have been very rapidly learnt, although no doubt one could argue that the biggest lesson of all—don't live in regions prone to earthquakes—has yet to be assimilated.

The United States represents, in very crude terms, much less than a thousandth of the world's earthquake fatality potential—first, because a relatively small proportion of the world's active fault system is located in the United States; second, and more important, because awareness of the earthquake hazard is higher and thus elementary defensive measures are more widely practised, particularly in construction. Few people die from the shaking itself; it is the falling of buildings, the collapse of dams, the starting of fires and so on which claims life.

There is a worldwide interest in the earthquake prediction programme in the United States (completely over-shadowing the arguably more interesting and important programmes in the Soviet Union and China). This interest is bound to grow with the publication of *Earthquake Prediction and Public Policy*, an extensive document from the Panel on the Public Policy Implications of Earthquake Prediction under the chairmanship of Professor Ralph Turner, a sociologist from the University of California, Los Angeles. The layers of bureaucracy in Washington are so thick that initials must suffice to describe the report's parentage and destination: it was prepared by the PPPIEP of the ACEP, CSS of the NAS/NRC for the FDAA of DoHUD.

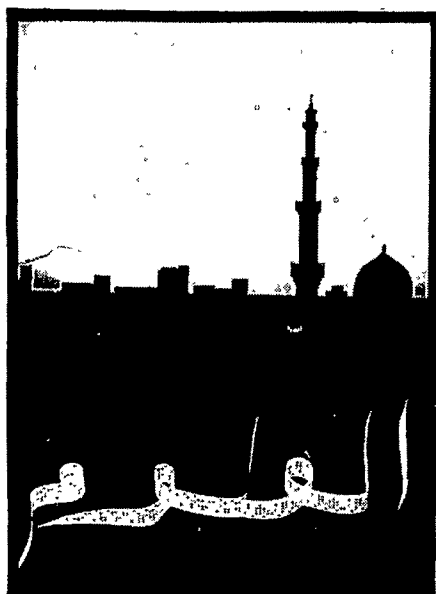
Predicting the reaction of the public to an earthquake warning seems to be every bit as difficult as predicting the earthquake itself, and this, no doubt, contributes in great measure to the unmemorability of much of the report. No stone could be said to have been left unturned, even down to expressing concern that tourists be alerted and jewellers empty their shops. The consequences of a prediction could be enormous; evacuations, unemployment, political in-fighting, demands for more money for emergency services, nuclear reactor shutdowns, draining of dams, attempts to withdraw insurance cover, failure to keep up mortgage payments, political attacks on scientists, local/state/federal squabbles, profiteering in property sales, demolition of buildings. The consequence of error is appropriately large. Small wonder then that it has already been said by Garrett Hardin that prediction could be more devastating than the event itself. The committee's first

recommendation is that "The highest priority . . . should be . . . saving lives, with secondary attention to minimising social and economic disruption and property loss, *provided the costs of specific measures are within the limits that society is willing to accept*" (our italics). Quite apart from the vagueness introduced by the second half of the recommendation, it is entirely possible that the social and economic disruption and property loss from the prediction will greatly exceed that from the earthquake, and might counterbalance the benefit from lives saved.

The panel did not find this very acceptable; it was passed off as "a popular theme currently espoused among some scientists and science popularisers". Another idea that it didn't like was that scientists might desist from making public pronouncements, at least for a few years. "Earthquake prediction is a fact at the present time . . . attempts to suppress information concerning premonitory signs would certainly fail—as they should". Now this is half-truth. Many scientists with the best will in the world towards prediction are looking very carefully at the limited evidence available and still wondering not only how universally applicable predictive phenomena may turn out to be but also whether the quality of the data that will be accessible without an enormous investment of cash will be sufficient to give unambiguous warning in more than a very few zones. And which zones are to be favoured? There are, in California, as many major earthquakes off the San Andreas Fault as on it; no-one foresaw the San Fernando Valley as a danger zone in 1971. Earthquake prediction is a fact in much the same way that travel to the moon is a fact.

Further, it is only half-true to say, as does the report, that "there is no way to monopolise prediction capability" (and thus to prevent predictions being made). The tools required for prediction include access to a whole range of data libraries, and were the government to impose a moratorium on public announcements while seismologists were given time to conduct more thorough research, it is not at all clear that any independent agency would either wish or be able to jump the gun. There might be a general welcome in all communities, scientific, governmental, legal, insurance, constructional for a long pause before prediction was permitted. The spectacle that this report portrays is rather the opposite; of a well meaning panel riding a small band of experts as fast as possible towards a confrontation with nature.

On two counts, then, this report gives cause for concern: that scientists may be rushed, and that the case against making a major response to a prediction may not be adequately examined. And Turkey's misfortunes underline yet again how little even elementary information on earthquake proofing crosses national frontiers. □



Eden with oil wells

The stereotyped image of the oil-rich countries of the Arab Peninsula is that of an artificial, fragile, air-conditioned Eden made possible by the purchase of Western comforts, technology and scientific background. The picture holds an implied threat of collapse by the time oil reserves become depleted which, by most reckonings, should happen sometime during the next century, perhaps in the first half of it. That image is, however, only partly true. Large investments are being made in an attempt to achieve what should amount to a leap from the Middle Ages to the atomic era. On the Arab Peninsula, schools have blossomed and primary education has, in most cases, become compulsory. Technical training facilities have been established, medical schools are about to produce their first graduates and, in Saudi Arabia, one of the world's most modern specialist hospitals has opened its doors. Alexander Dorozynski reports on science and technology on the Arab Peninsula, where the avowed aim is to achieve scientific and technical self-reliance as rapidly as possible, and which, if the present effort is maintained, may well become the site of a unique achievement.

ALTHOUGH per capita income in several countries of the Arab Peninsula is among the highest in the world, income as a product of labour is among the lowest. Wealth has not had time to filter down to the majority of the population, and rich countries such as Saudi Arabia and Kuwait are still developing nations. But the means for rapid scientific and technological progress essential to development are available, and it seems that Arab leaders have understood the need to provide for future generations.

Thus, not only is there an effort to transfer Western technology, but to revive a scientific tradition that has largely been dormant since the days when the Arab empire extended from Spain to Samarkand, when cultured Arab envoys expressed surprise at the illiteracy of Charlemagne's court, and Arab scientists helped pave the way for the European Renaissance.

In Saudi Arabia, for example, illiteracy was widespread only 20 years ago, and the only centre of higher learning was the Islamic Studies Faculty, founded in Mecca in 1947. But since the University of Riyadh was founded in 1957 with a teaching staff of nine, the situation has changed rapidly. The College of Petroleum and Minerals (CPM) was set up with the help of the Arabian American Oil Company in 1963, and the Abd al-Aziz University was started in 1967 as a private institution sponsored by a group of business and political leaders.

Now Riyadh University has an enrolment of 6,000 students (about as much as the 100-year-old American University in Beirut) in eight faculties: arts, education, sciences, business, pharmacy, agriculture, engineering and medicine. The Faculty of Medicine, is provided with professors, advisers and examiners by the University of London, and it will be the first faculty to move, in three or four years' time, to the \$500 million campus now being erected on the outskirts of the city. (In fact there will be two campuses, one for men, one for women.)

The Faculty of Sciences publishes a staff bulletin, whose contents reveal the wide range of interest and varying degrees of sophistication of research projects. A single issue, for example, contains a fairly simple and straightforward description of the morphology of the grasshopper and an analysis of climatic elements in the kingdom (with emphasis on potential agricultural developments), but also reports on subjects such as the electron spin resonance of several compounds. Professor A. A. Al-Khayal, Dean of the Faculty of Sciences, already voices a complaint familiar in academic circles, although unexpected in Saudi Arabia: lack of funds for facilities and

laboratory equipment.

The CPM, moving to an elegant, futuristic looking campus and chaired by Sheikh Ahmed Zaki Yamani, Minister of Petroleum and Minerals, teaches sciences, engineering science and applied engineering to some 1,000 students. High standards of admission are maintained by professors who, for the most part, come from American universities. The Abd al-Aziz University (arts, sciences, business and administration) has been integrated into the kingdom's educational system, and now has nearly 3,000 students.

These numbers alone, for a country of some 6 million people suddenly emerging into the modern world, represent an achievement. Another is the fact that women, traditionally kept outside the mainstream of life, now have access to primary and higher education. (This is attributed to the late King Faisal himself, and the influence of his wife, Iffat.) More than 200,000 girls, as well as half a million boys, attend school, and the numbers are constantly growing. Riyadh and Abd al-Aziz Universities have special teaching facilities for women. There is a shortage of women professors, and in many cases female students in separate quarters follow courses on closed-circuit television, and ask questions by telephone. Education at all levels (as well as medical services) is underwritten by the government.

Foreign observers familiar with Saudi institutions of learning generally agree that the level of standards is good and improving rapidly, particularly in professional education. But a straightforward comparison with Western institutions, which benefit from centuries of academic background and where religious and traditional values play a less important role, is difficult. Nevertheless, the admission of Saudi graduates as doctoral candidates at such institutions as MIT and Caltech is indicative of the standard that can be achieved.

An interesting (and controversial) initiative in Saudi Arabia has been the creation of the King Faisal Medical City and Specialist Hospital, which was inaugurated in April (before the graduation of the country's first crop of medical doctors). Conceived by King Faisal himself, the project was carried out under the leadership of Dr Rifat Alsayed Ali, personal physician to King Faisal and the royal family, who has coordinated the activities of dozens of foreign companies which have contributed to the building and equipping what is to become one of the world's most modern in-patient and out-patient specialist hospitals, offering facilities and skills seldom available under a single roof. The 250-bed hospital will be operated by an inter-

national staff of 750, part of which has already been contracted for. Future capacity will be 500 beds, with a staff of 1,200. Later this year, a clinical research department will be opened.

The idea, says Dr Ali, is not only to provide on-the-spot diagnostic and treatment facilities that many wealthy Arabs may seek in Boston, London or Paris, but to seek rapid medical progress in the region with the help of top foreign experts for whom the best possible working and living conditions are being provided.

The hospital has started operating on a limited scale, and on a paying basis. "Otherwise", says Dr Ali, "everyone will want to come here rather than to another hospital. But if any patient requires facilities and specialist treatment available only here, he will be admitted as a free patient if he cannot pay."

Another ambitious project in the kingdom is the development of agricultural research, considered as essential to self-reliance; the recently constructed laboratory of the Ministry of Agriculture near Riyadh is one of the most complete in the world in its design and equipment. It has yet to be fully staffed.

In Kuwait, where the development of a welfare state was started in 1951 by Sheik Abdallah as-Sabah and continued by his son and successor, Sheik Sabah as-Salim as-Sabah, schools have proliferated to provide for now compulsory primary education, and there is a splendid university (with a campus for women) which has research facilities with the latest equipment.

The progressive integration of women in a man's world is, here, more evident than in Saudi Arabia, where even secretaries are male. In Kuwait, women secretaries wearing modern dress are a common sight, and female researchers go unnoticed.

The Kuwait Institute for Science Research (KISR) has been operating for five years, and its prime purpose, in the words of its director, Mohammad Al Shamali, "is to build up a tradition for research. It is the first step, and perhaps the most difficult one to take."

While pursuing this goal, the KISR concentrates on research projects particularly suitable and useful to the country. Dr Al Shamali has long been interested by the potential use of indigenous desert plants, and a multidisciplinary research team led by Ibrahim Hamdan, an American-educated food scientist of Palestinian origin, is now trying to determine their nutritive value and their potential toxicity.

There are several hundred varieties of desert plant, perennial, annual and bushes, and little is known about them except that a little care, and a little more water, can greatly increase their

ONE of the most important catalysts to development in the Middle East is the Kuwait Fund for Arab Economic Development, created in 1961 as a government agency but granted complete independence in its finances, organisation, and policies. As Abdlatif Y. Al-Hamad, its Director General, likes to point out, one of its first principles is neutrality with regards to political and social systems adopted in the Arab countries. This neutrality seems to be more than simply token, as the fund has supported projects in countries with widely different political systems, such as Southern and Northern Yemen, Syria, Tunisia, Egypt and Mauritania.

Until last year, the Kuwait Fund functioned essentially as a regional development bank, providing assistance chiefly in the form of loans on concessionary terms, with interest rates around 3 or 4%, maturity about 20 years, and grace periods generally corresponding to the time needed for the completion of a project. (Some grants were also given, notably for technical assistance and the financing of technical or economic studies.)

In July 1974, the fund took a new turn when the National Assembly extended its operations to the rest of the developing world, and increased the declared capital from KD 200 million to KD 1,000 million, that is, more than \$3,000 million. Since then the fund has undertaken to support a number of development projects in Africa (livestock and sugar industry development in Uganda, river basin development in Senegal, tea plantations in Rwanda, textile spinning and weaving in Tanzania, construction of two harbours and a railway in Gabon)

Other projects are being studied,

and the fund's director general has visited Asia, where several projects are expected to receive financial support (a urea plant in Sri Lanka, sugar factories and land reclamation in Afganistan, irrigation in Bangladesh, land rehabilitation in Malaysia).

In 1971 the Kuwait Fund was followed by the Arab Fund for Economic and Social Development, with headquarters also in Kuwait. The Arab Fund's charter restricts its activities to Arab countries, where joint developments are planned in such fields as highway transportation, telecommunication and agriculture. Finding that there is a shortage of well-prepared national or regional projects, the Arab Fund has undertaken its own studies to help the development of a country in its regional context.

There doesn't appear to be an 'OPEC strategy' concerning aid to developing countries, but many OPEC members have reacted to the often-voiced charge that increased oil prices have crippled the development of poorer countries. Thus India has benefited from credit terms to obtain oil from Iran, Iraq and the United Arab Emirates, and for the poorer African countries, Arab oil producers have created a \$200 million fund to offset higher oil prices. Joint ventures have also been undertaken between rich and poor countries, for instance, between Kuwait and Mauritania to develop iron mines.

As there is little coordination between the various forms of aid financed by the OPEC, total figures are difficult to come by, but one estimate is that last year, more than \$5,000 million was committed to developing countries, either directly or through international agencies.

growth rate. Some of them have a good protein content, and, in a region where most of the fodder for cattle is now imported at considerable cost, they represent a potentially important natural resource.

A first step in the research project has been the identification of the principal varieties, some of which also exist in similar semi-arid regions of Australia, South and North America, but about which data is scattered or incomplete. This is now being followed by a determination of their nutritive value: a more sophisticated analysis of their carbohydrate, protein and crude fibre content. According to Ibrahim Hamdan, one perennial plant, atriplex, has been found to have a protein content of 16%, as high as that of alfalfa.

Many of the plants have been grown on the grounds or in greenhouses of

the KISR, and it has been shown that some of them may be suitable as a local substitute for more classical grazing plants that are costly to grow in the semi-arid tropics. Plants from regions with similar soil and climatic conditions have been included in the study, and attempts are being made to create a symbiosis between some desert bushes, annuals, and perennial plants (for instance, a deep-rooted shady bush, a climate-resistant perennial, and a succulent annual).

Recently, the cattle-feeding experiments were started on a desert plot of 20 square kilometres, acquired by the institute, and ways are being sought to complement this roughage with locally available additives, such as single-cell 'petroleum proteins' or microscopical algae (which are also experimentally grown at the KISR).

Toxicity studies have allowed the project to branch out into the study of the potential medicinal properties of some of the plants. Ali Anani, a chemist, has identified a number of alkaloids and other substances that are being studied in the particular context of traditional Kuwait medicine, which itself is probably an offshoot of ancient Arabic medicine, still practised by healers (*attarin*) who coexist in Kuwait with the free, Western-style medical services provided by the state. The *attarin* use herbal medicines, but jealously guard their secrets, sometimes mixing a number of substances in their preparations, apparently to camouflage the active ingredients. Some of the plants have already been shown to possess antibacterial activity. Soil bacteria and fungi have been included in the study, as well as hormone-like promoters of plant growth and soil enzymes that could be used to improve fibre digestibility.

Dr Mohammad El Shamali and Dr Hamdan believe this research may well turn out to be of significant importance in the development of agriculture, but also that it is a good way to train researchers of varied disciplines and nationalities to work together as a team.

It would be meaningless, again, to compare research undertaken in Kuwait with that of an MIT, Rockefeller or Pasteur Institute. But the effort is obvious, and the results, considering the lack of research background and tradition, encouraging indeed.

The pattern is repeated elsewhere on the peninsula. In the small state of Bahrain (where oil was found in the early 1930s) there are more than 100 schools for a population of 220,000, and several vocational training centres. In tiny Qatar, there is a technical school, a school of commerce, a teacher's college, and a total of about 90 schools; last year, the ratio of teachers to the population was about 1 to 1,000.

At the same time, the 'technological transfer' continues, with an increasing amount of goods being produced on the spot (albeit with the help of foreign technicians) rather than imported. Thus, while a few years ago the story was that a desert prince would discard his Cadillac when the ashtray was full or the tank empty, now there is a plant in Saudi Arabia where cars are overhauled and rebuilt, and arrangements are under way to set up assembly plants. In Riyadh, production of pharmaceuticals is expected to start this year; the General Petroleum and Mineral Organisation (Petromin), a public corporation owned by the state, runs a steel rolling mill on the Red Sea coast south of Jiddah. Local

THE government of Saudi Arabia is planning to spend \$12,000 million of its oil wealth in an effort to turn the country's desert green.

The desert lies 180 miles east of Riyadh and is the centre of a massive desert reclamation programme, part of a \$143.5 thousand million five-year development plan.

Altogether, the Saudi Arabian government plans to turn 4.18 million hectares of sand into farmland, which according to the Under Secretary of Agriculture, Mr Taher Efeid, will take at least half a century to accomplish.

At present, only 1.4 million acres of farmland are cultivated in the desert monarchy. This earns more than \$25,000 million in revenues annually.

The Kingdom envisages improving and regulating underground water resources and installing an efficient drainage network to reduce salinity in various areas.

Five research centres, dealing with fishing, insecticides, fodder, seeds, fertiliser and poultry have been set up in Jeddah, Riyadh, Jassa, Hofuf and Qatif. These centres also operate model farms.

Two hundred and fifty types of rice are being tested to cultivate 167,000 acres which will raise the production from 75,000 tons to 100,000 tons over four years.

To encourage private investment in agriculture, the government will distribute reclaimed land at an average of 32-160 acres per farmer. The farmers will be given three years to prove they are capable of tending the land satisfactorily. Otherwise, the land will be taken and given to

another more experienced farmer.

In the incentive programme, farmers will be able to purchase agricultural machinery at 55% of regular prices

The prices of imported fertilisers will be reduced by 50% and dairy equipment will be sold at 70% of their import prices.

Farmers will receive \$2.50 annually for each sheep and \$14 a year for every she-camel.

There are 17 medium-sized dams in the country and it is hoped gradually to increase these to 23. Most of the dams control rain waters in the southern region of Jaizan and Abha.

The biggest—Jaizan dam—is approximately 1,000 feet long and 125 feet high with a capacity to store enough water to irrigate 50,000 acres. It cost \$27 million to build.

Seventy million dollars were spent on the Hassa irrigation and drainage programme which increased the cultivable area around Hofuf from 20,000 acres to 50,000 acres.

Also under the plan \$51.8 thousand million was budgeted for electrification projects, desalinated water production is expected to rise from 57 million gallons a day to 163 million and a total of 270,000 new homes—some in reclaimed areas, are to be built.

Agriculture officials say that 'the days of scratching a living from small sandy plots are over for the farmers and Bedouins of Saudi Arabia'.

Vegetable production is near self-sufficiency, reports say, and the farmers who had deserted their salinity stricken farms are now returning.—*Gulf Mirror*.

cement factories supply about half of the demand of a booming construction industry; fertilisers and polyethylene bags to store them in are made on the spot.

In Bahrein, aluminium ingots are made from imported bauxite and exported. Kuwait has an aluminium smelter, clothing and household goods factories, plants making prefabricated houses and building materials. Research in hydroponics has been going on for several years, and some vegetables produced in this way now reach the market in Kuwait city.

Although one does not find on the peninsula the relatively sophisticated native technology such as is frequent in Beirut, Amman or Cairo, it should be remembered that only a few years ago, the Arab Peninsula was quite literally a scientific and technological desert. An added impetus to this awakening comes from collaboration with the poorer Arabs—Egyptians, Jordanians, Lebanese, Palestinians—

and an effort is being made to attract from Europe and particularly from the United States Arab scientists who have become established abroad, and even taken foreign nationalities. Most come on short-term contracts (a sabbatical year for instance) but a few find their ancestral land so transformed that they are tempted to remain.

There is, understandably, a problem posed by instant wealth. Although it has certainly given a boost to this 'leap' into the modern age; sometimes it becomes an obstacle by removing some of the incentive to learn in a system where not only is there no tuition but nationals receive a substantial living allowance. It seems, though, that there is a consciousness of this, and in several centres of higher learning, incentive have been set up to reward effort and achievement. Talented young Arab graduates are subsidised to continue their studies abroad, and are assured of finding a rewarding position when they return home. □

international news

MEMBERS of the House of Representatives returned to Washington from their summer vacations last week and promptly passed a bill which, according to its supporters, may save the United States as much petroleum as the entire supply from Alaska's North Slope. The key to that amazing prediction is the successful development and mass production of an electrically powered car—an idea which has been batted in recent years by plenty of rhetoric but few dollars.

The bill would provide as much as \$160 million over the next five years for a research and development effort designed to improve battery technology and to pave the way for commercial introduction of electric vehicles on a grand scale. That amount should be compared with the trifling sum of \$1.166 million which was spent by the federal government on battery-powered vehicle studies between 1969 and 1974.

Battery-powered cars are, of course, already in commercial production, but their range is limited to about 50 miles because they use conventional lead-acid batteries, which need frequent recharging. Nevertheless, as one proud owner of an electric vehicle, former Atomic Energy Commissioner Clarence E. Larson, pointed out in a letter to the *Washington Post* last week, 50 miles is quite far enough for most city trips.

The potential market for electric cars is certainly large. For one thing, it is estimated that 50% of all car journeys in the United States are less than five miles, which says a lot about unnecessary use of the automobile. A more pertinent statistic is that about 98% of all trips taken with second family cars, lie within the 50-mile range of present battery technology. The second car, which is part of the American way of life, is therefore clearly the target of the electric car industry if, or when, it is in business.

Other advantages claimed for electric cars are that since they will mostly be charged up overnight, when electricity demand is low, no new electricity generating capacity will be required. It also goes without saying that the lack of pollution from the car itself will be a good selling point. And much is also made of the fact that transportation now accounts directly for about 25% of total energy use in the United States; the potential saving in imported oil by replacing petrol-driven cars with electric vehicles is

Congress boosts electric car research

by Colin Norman, Washington

therefore immense. (But it should be noted that total energy savings will be negligible since the energy required to produce the electricity will offset the savings in petroleum. The important point, however, is that the net effect will be to shift energy consumption away from oil to coal and nuclear power.)

But the picture is not quite as bright as the most ardent enthusiasts maintain. Political, technical and economic problems could all stunt the growth of the fledgling electric car industry.

First, the costs. A study carried out by the Environmental Protection Agency, which was published last October, concluded that electric cars would be between 20 and 60% more expensive overall than equivalent conventional cars until battery develop-

ment significantly reduces battery depreciation costs. The expense of charging up the battery is only a part of the total operating costs, and some technological advances will be required both to extend the capacity of batteries and to make them more durable.

As for the range of electric cars—probably the chief commercial drawback of present models—a committee of the National Academy of Sciences estimated in 1973 that it would take at least four years, assuming adequate funding, to develop an advanced battery power plant with a range of over 100 miles. The committee estimated that it would then take an additional seven to 10 years to mass produce cars with advanced batteries. Those time scales are now considered to be optimistic, however, largely because private and public funding has not built up as rapidly as expected.

Finally, neither the automobile industry nor the oil industry have shown much enthusiasm for the electric car. Both are powerful lobbies and according to some observers they have been effective in holding up the development of battery technologies. Whatever the truth of allegations, it would certainly be a major operation for Detroit to

Pasteur Institute on way back to solvency

AFTER reviewing a report of the Pasteur Institute's financial status, Madame Simone Veil, Minister of Health, has persuaded the French government to increase credits allocated to it from some 20 million francs in 1975 to 50 million francs in 1976. This is in addition to subsidies to the institute's teaching activities, and to total or partial salaries paid to some Institute researchers by the Centre National de la Recherche Scientifique and the Institut National de la Santé et de la Recherche Médicale.

The new budget is to be approved by the parliament, but it has already been announced by the Ministry of Health and, barring the unforeseen, should be passed.

According to Dr Joel de Rosnay, director of development, the grant will ensure solvency, and enable the institute to continue its development following a businesslike reorganisation earlier this year. It covers about half of the expenses of the foundation, the research branch of the institute. The

rest will come from other resources, including interest on capital, donations, and income earned by Institut Pasteur Productions, the institute's commercial arm. (A forthcoming innovation will be the standardisation of the "mutatest" devised by Dr Bruce Ames of the University of California at Berkeley. The test relies on the ability of carcinogenic chemicals to cause mutations of strains of the common bacteria *Salmonella typhimurium*. Developed in France in collaboration with the International Agency for Research on Cancer in Lyon, it will be available commercially).

In addition, during the recent visit to France of Sheikh Sabas Al Salem Al Sabah, the Pasteur Institute received from Kuwait a donation of 5 million francs, about half of the institute's annual deficit. This donation will be used "out of budget" to finance research on infectious and tropical diseases, embryology and cancer.

—Alexander Dorozynski

Four years after its creation, the Canadian Ministry of Science and Technology is to be reorganised. News of the change was given by the Minister of State for Science and Technology, Mr C. M. Drury, at the thirteenth Pacific Science Congress. "The ministry's job has been to advise the government on how science and technology can be used and developed to the best advantage of Canada", he said. "This is a difficult task and we have learned much about the capabilities of such a horizontal ministry in the four years of its existence. Based on this experience the ministry is now being re-organised in order to enable it to function more effectively."

It will be organised at the working level to communicate with the three principal segments of the scientific community—universities, industry and government and will include the many professional and learned societies. The ministry's policy-making role was defined by Mr Drury in two categories: major continuous policy assignments with long-term implications, such as development of a national capacity to study and assess the impact of science and technology on society; and development of policies and programs for the co-ordinated and large-scale use of scientific resources beyond the scope of a single agency or government, such as the oceans policy.

"The job of the ministry", said the minister, "is not to displace or duplicate the roles of existing departments and agencies in science, engineering and technology. Nor will we try to become a sort of national oracle for science, issuing decrees on what scientists in government should and should not be doing."

● A discussion on Canadian science policy showed how far apart the bureaucrats and ordinary scientists are. While the Minister outlined precisely what science policy meant to the government, the Dean of the University of British Columbia's Faculty of Graduate Studies, P. A. Larkin, declared himself confused and be-

wildered by the concept itself—and abashed by the way in which it was being carried out. And as for communication on the subject between scientists and government, he said: "You have about as much chance of getting through as if you were reciting Gaelic poetry to a deaf seagull."

Larkin called his address "Ask Archimedes", because, he said, interest in science policy goes back a long way: "For example, Archimedes used his talents in many ways in the service of his native city of Syracuse, particularly in times of war, and presumably the king of Syracuse thus had a 'science

Science in Canada

from David Spurgeon, Ottawa

policy'—which was, 'Ask Archimedes'."

On the other hand, said Larkin, Canada's science policy for 50 years was "me too", meaning that it simply copied the policies of other countries. "And its implementation was accomplished when C. D. Howe, the 'minister of everything', played golf with C. J. Mackenzie, who, as the President of the National Research Council, was the architect of the national determination to build scientific intellectual resources."

Later, Larkin continued, many reports were published on the national scientific effort "Virtually every report by a committee or task force on science policy in the last 20 years has had something to say about the failures in coordination, communication, and integration; and the frightful sins of duplication, procrastination, and bureaucratisation. Since the most recent reports sound very like the older ones, it seems likely that either no one reads the reports or, if they do, they pay little attention."

Outlining some of the main thrusts of science policy thinking, Larkin declared himself—and others—be-

wildered. It has become obvious that so many changes are taking place so rapidly, he said, that no one has a good grasp of where we are collectively headed. "Even if someone could see it all clearly, it is most unlikely that he could do anything about it. World wide, science and technology are in a state of extremely rapid and turbulent evolution. There are so many scientists, and communications have become so good, that problems are perceived, attacked, solved and the solutions applied almost before most of us are aware of them . . . Science policy has become a curiously all-pervasive and eclectic exercise in which it is increasingly difficult to judge whether you are worrying about the right thing."

Furthermore, said Larkin, most reports concerned with science policy are obsolete by the time they are published. Few read them any more. Most are "essentially a condensed summary of a consensus that has not only been reached but partly implemented before the report is made public. With several such studies going on at once, the national capital often gives the impression of being a full time continuous scientific conference in which everybody is writing papers and no one is attending the sessions at which the papers are presented."

The committee, task forces, commissions and the senior Civil Service, said Larkin, "move along close to the margin of what almost seems uncontrollable change, documenting and formalising what has already happened, arguing a bit about who thought of it first (when none of them did), performing the apparently essential tasks of administering and reorganising, but in reality never realising they are like the bubbles in beer, produced by pressures that are quite incomprehensible."

Mr Drury called Larkin's comments about communication between scientists and government "cynical", and said that some institutions and mechanisms already existed for such communication, and that one task of his ministry was to devise other methods

switch some of its production to an entirely new automobile concept. The big car makers have, after all, raised enough complaints about the relatively minor production changes necessary to meet air pollution standards.

Those obstacles to commercial production are the chief factors which prompted the House of Representatives to approve massive federal support for a research and development effort last week. The bill, which was largely the work of Representative Mike McCormack, instructs the Energy Research and Development Administration to

devote more money to developing advanced battery technology. But the heart of the measure is a demonstration programme through which the federal government will underwrite the costs of at least 7,500 electric cars during the next five years.

The purpose of the demonstration programme, according to a report written by the House Science and Technology Committee, "is to get present and future state-of-the-art electric vehicles out into every region of the country". They will be used in federal, state and local government fleets, by

businesses and by individuals, to gain experience of driving performance, maintenance costs and so on. An important aspect of the operation, however, is that it will provide an incentive for industry to set up the production facilities, which should pave the way for mass production later.

The bill was passed easily by the House—by 308 votes to 60—and the Senate Commerce Committee will hold hearings on a similar measure early next month.

It is expected that the Senate will also pass it easily. □

correspondence

Good for science?

SIR,—Your editorial "Good for people, bad for science" (July 10) requires a prompt comment, for you apparently advocate a major change in the system of academic tenure. At the same time, your editorial seems to indicate neither an awareness of the complexity of the issue, nor of experience with alternative tenure systems, nor of the considerable literature in this area; I refer here primarily to the tenure system that is very widely used in the USA, the role of the American Association of University Professors (AAUP), and the many articles and books in which this subject has been discussed.

Tenure is generally awarded, in US colleges and universities, after a probationary period that should be long enough to provide sufficient time for a reliable assessment of the capabilities and promise of a faculty member, yet should not be so short as to lead to an unacceptable proportion of bad decisions. The AAUP guideline of a maximum probationary period of seven years is widely followed, but universities will often award tenure much earlier in an attempt to hold on to younger people of exceptional ability. This does not, of course, ensure that all those who have tenure will continue to merit it, and removal for cause (including demonstrated incompetence, as decided by an elected faculty committee) is recognised, though rare.

The major benefit conferred by tenure is the freedom that faculty members should then have to study, discuss and explore without fear, views and topics that may be highly unpopular or controversial at that time. Most scientists pursue careers that are models of conformity and caution, but some may wish to be more adventurous. Research involving human subjects raises strong passions—consider the continuing debates over foetal research and genetic engineering. If these scientists were subject to renewable contracts, it is safe to predict that, for many, caution would increase as the renewal date approached, and topics that may seem of major scientific interest would be neglected if adequate protection could not be guaranteed for those who wished to work on them. These are not hypothetical fears of imaginary but unlikely situations: these pressures on the academic community exist and should be recognised.

There is another serious objection to the proposal for contracts: who would review them and make the decisions on renewal? A senior administrative officer in the university or in Whitehall? With what advice? Or a group of faculty members or the department chairman? One may reasonably claim that professional competence can only be judged by others in the same discipline, but this is more likely to lead to leniency on the part of the reviewers in the hope of similar treatment when their own renewals come up. Leaving the decision to a department head carries other risks: renewal will provide an excellent opportunity to get rid of the abrasive members of the faculty. Indeed, extensive AAUP experience has shown that a major proportion of the threats to faculty members come from other faculty members and from department heads and deans who were once also faculty members. Overall, the apparently attractive idea of renewable contracts bristles with severe problems of implementation, and should not be lightly suggested without at the same time treating this side of the suggestion.

It is not only on the frontiers of research that feelings become inflamed and faculty positions are threatened. Some of us hope that more university scientists will become engaged in what Ravetz has termed 'critical science' (*Scientific Knowledge and its Social Problems*, Oxford University Press, 1971). This involvement in current issues should not need to be partisan but can be in the direction of elucidating complex technical issues to a broader public. Here again, these scientists need the protection of tenure, for, it is clear, those employed in industrial and government laboratories do not have the freedom to engage in these issues unless they are generally on the side of their sponsors. University science departments will probably provide virtually the only source of independent comment, and these scientists will not be able to play a responsible role if their jobs are in jeopardy. Here again, this is not some hypothetical possibility: as 'critical' science has slowly developed in the USA, government and industrial scientists have been conspicuous by their silence.

Those of us who have had experience in faculty matters, with tenure and with the AAUP, recognise that the weakness of the tenure system is the

protection it extends to those who prefer to relax and do little or nothing, but most of us consider this is a modest price to pay for the many advantages. A tenure system can operate no better than those who have to make the decisions; some decisions will be good and others bad. A recent thorough examination of the tenure system (*Faculty Tenure*, Commission on Academic Tenure; W. R. Keast, chairman; Jossey-Bass Publishers, 1973) produced a strong endorsement.

In summary, then, your editorial is disappointing. Perhaps the British universities' tenure system does need overhaul; perhaps a probationary period similar to that in the USA should be considered, along with a greater involvement of the non-professional faculty in matters of faculty promotion and tenure. But I would hope that notice is taken of the extensive and documented experience before adopting a contract system that has a superficial appeal but is potentially severely detrimental to academic freedom.

Yours faithfully,

M. W. FRIEDLANDER

Washington University,
St Louis, Missouri 63130

Fellows overseas

SIR,—I can well understand the frustration felt by UK postdoctoral fellows overseas as described by Richard James (August 7). Since I doubt that the "structure of the UK University system" will change, I can perhaps do a little to put the position straight, at least as I see it from my department, and make a suggestion.

All of us want very much to appoint the best people to the few lectureships available. Our system of advertising the posts as soon as we are assured of the finance is not only efficient but is fair and should not be classified as ponderous. It would unnecessarily delay matters to set a deadline for receipt of applications which gave time for the advertisements to be seen by all potential applicants overseas. In my experience we never keep strictly to the deadline, and will always consider late applicants and give special consideration to those who are overseas. A brief airmail statement to the effect that a formal application is on the way is certainly helpful. In short I do not believe that the heads of departments handling the regrettably

small number of lectureships are unsympathetic and ponderous.

I agree that it would be better if we could all plan appointments much further ahead than we do at present. I think this is unlikely to happen in the foreseeable future. May I suggest, therefore, that it would be very helpful if overseas postdoctorals who are interested in returning here keep in touch with their former head of department and, more important, make an arrangement with a former colleague who would provide information concerning any vacancy that might be of interest.

Yours faithfully,

P. N. CAMPBELL

Department of Biochemistry,
University of Leeds, UK

Engineering standards

SIR,—In "Round Britain (July 31) you report, and seem to approve, the Committee of Vice-Chancellors and Principals' pressure to increase the academic standard of British engineers by encouraging employers to insist on higher degrees. My job is engineering design and project management in an advanced field (fast reactors), and I can sympathise with the CERN engineers who despair at the often woefully inadequate capabilities of British industry; but I should need a lot of convincing that this has anything to do with the number of years people spend at university. Most would agree that where fast reactors are concerned the ranking order of national success is France, USSR, United Kingdom, with USA, Japan and West Germany following somewhere. I am pretty certain that this does not correlate well with the national proportions of engineers holding higher degrees, but perhaps the vice-chancellors would like to give us some statistics?

Success in advanced engineering projects obviously demands a reasonable level of theoretical ability, but it depends much more on the attitudes and policies prevalent nationally and within industrial firms, and on the ability of engineers to handle unfamiliar and very complex technical and organisational problems with imagination and good judgement. I suggest that to encourage large numbers of men to spend two or three years calmly researching esoteric problems is not likely either to improve government and public attitudes to engineering or to develop the necessary flexibility in the men concerned.

For my money I would support the Open University against all the dignity of the vice-chancellors; its technology courses are designed to stimulate thoughtful, imaginative and socially responsible attitudes. It is being squeezed financially by the government

that conceived it, and it is also not yet receiving the recognition it deserves from professional bodies. In these circumstances to channel extra resources into expanding post-graduate engineering departments would be short sighted indeed; what is needed in British engineering is more excellence and public acceptance at first degree level, not more narrow specialisation.

Yours faithfully,

JOHN A. GATLEY

Knutsford,
Cheshire, UK

All at sea

SIR,—Recently (May 8) you reported on some of our work in your editorial "For those in peril on the sea". Scientists and crew aboard research vessels co-exist under tension, created mostly by the needs of scientists and felt mainly by crew; tension which can and does erupt into unpleasantness. This costly problem is now recognised by the Canadian and German governments, for example.

Our most basic finding is that this situation results from locking two warring sub-cultures—academicians and people of working-class orientation—on a ship at sea where they cannot avoid each other as on land. The single most important cause of tension is the "data hunger" of scientists and their degree of sensitivity to the relaxation needs of the crew.

Many scientists, yourself included, have criticised our findings. Unfortunately, they all did so having read only a news release (which mentioned only Bernard). Published work should be read before criticism. We question your saying, "for half the price of placing an anthropologist . . . (you) would have been happy to tell" ONR about shipboard life. The total cost was less than three days' 1972 ship time (and covered part of a larger project to describe human relations numerically).

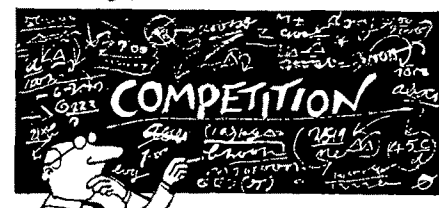
It is important for scientists to realise that mariners' views differ intrinsically from your editorial. For example, you compare the chief scientist with a referee, and believe that captains always want to get ships to port a day early. But captains also see themselves as referees, and believe that scientists always want to get ships to port a day late. Whether any of these sentiments are true (in fact ships do dock both early and late) is irrelevant. The important thing is that these sentiments are believed by those concerned, and insensitivity by others to these beliefs causes conflict.

Anthropologists rarely learn much that the natives, like yourself, do not know. Your observations affirm some of our work. We hope to hear from

other members of the ocean science community on conditions aboard any nation's vessels. A dialogue might help prevent Bransfield-like incidents.

PETER D. KILLWORTH

Cambridge, UK



RESULTS of competition No. 1. Readers were invited to supply the minutes of an illicit seminar held by scientists somewhere in the Western world. Though we withheld judgement for as long as possible, we were finally forced to recognise that there simply wasn't a welter of witty replies held up in the post somewhere. It may have been that the subject was too baffling for many to cut their teeth on. Winner from the half a dozen entries was E. Jarvis, of Clapham, London (entry below). A further prize goes to Scott Gilbert, of Johns Hopkins University, Baltimore, for a near miss.

AMSS in session

THE Anti-Metric Society of Scientists held a meeting at a secret location in Mile End Road.

It was resolved that they would not budge an inch in their efforts to resist metrication and that they would continue to fathom out ways to circumvent its introduction. The problems were more than pint-sized but there was still much mileage in their opposition activities.

Contingency plans were drawn up to ensure that members would at least obtain their pound of flesh; an issue would be made of diamond cutters for reducing litre glasses to pints and pocket saws for cutting down metre rules to yards.

The ladies' committee announced that Valentine Cards inscribed 'I love you a bushel and a peck' would be available to members by the dozen.

Competition No. 2. An easier test this time, with a longer time limit of six weeks to allow for mental as well as postal blockages. A prize of £10 awaits the winner (or winners):

There was a young lady called Bright,
Who travelled much faster than light,
She went out one day

In a relative way,
And arrived home the previous night.

Ms Bright and her épéeist companion Fisk are already immortalised in limericks with a scientific flavour. Competitors are asked to submit further examples based, in similar vein, on fundamental scientific principles, observations and so on. □

news and views

A YEAR ago *Nature* published a communication by J. B. Barbour (*Nature*, **249**, 328; 1974) on "Relative-distance Machian theories". There were some general comments in *News and Views* (*Nature*, **249**, 305; 1974), but no discussion of details because Barbour promised another model having 'more interesting properties'. This was recently published under the title "Forceless Machian dynamics" (Barbour, *Nuovo Cim.*, **26B**, 16; 1975). It may be useful now to comment on the model without again reflecting upon wider aspects of so-called Machian concepts. This is possible only with sufficient indication of Barbour's mathematical procedure, while keeping it as concise as possible; I concentrate on his recent paper, but rely upon the earlier one for certain explanations.

Barbour considers a system of N particles of mass $m_1, \dots, m_i, \dots, m_N$, with $\sum m_i = M$, where m_i is an intrinsic number associated with particle i . They are in a three-dimensional Euclidean space; any configuration of the system may be specified by a point in $3N-3$ -dimensional space (since only relative distances are to be significant) called relative configuration space (RCS). The history of the system is a curve in RCS; a suitable parameter along the curve defines time t . Barbour assumes that the history is got by making a Lagrangian function L extremal, and a necessary consequence of his interpretation of Machian requirements is that L is a function only of the distances r_{ij} between the pairs of particles and their t derivatives. For the system of simple particles he postulates

$$L^2 = \sum m_i m_j \dot{r}_{ij}^2 / r_{ij}$$

Now, however, Barbour is interested in replacing these particles by bodies admitting some internal motion. In order to disclose the consequent possibilities, he constructs a highly particular model; it is probably the simplest that is capable of exhibiting the features to be discussed—it is certainly ingenious.

For simplicity, the model is confined to a Euclidean plane; as before, it consists of a number of masses, but here each mass m_i is a ring of radius a and uniform line density ρ_i , where $m_i = 2\pi a \rho_i$, and $a \ll r_{ij}$ for all i, j ($i \neq j$) so that r_{ij} may still be called the distance between bodies i, j . The angular coordinate corresponding to rotation of ring i about its centre is ϕ_i .

As he says, Barbour proceeds to calculate a test-particle 'situation'—

Mach in detail

from W. H. McCrea

'scenario' in the vernacular. He considers a test-ring, also of radius a , of mass $m \ll m_i$, and with angular coordinate ϕ , where the rotational velocity $\dot{\phi} = a\dot{\phi}$ and the translational velocity of the test-ring will be found to vary with its location relative to the others, but m is so small that its effect upon the motion of the others is negligible.

At time t the test ring has polar coordinates r, θ relative to some particular ring S of mass m_s , which is assumed to rotate at constant speed $a\dot{\phi}_s = c$. Then if the appropriate part of the foregoing Lagrangian is expressed in terms of elements $ap d\phi$, $ap_s d\phi_s$ of the rings m , m_s and integrated over them, they make a contribution to L^2 , up to terms in $(a/r)^2$,

$$\{\frac{1}{2}mm_s(2\dot{r}^2 + \dot{\chi}^2 + c^2)\}/r$$

For the model, Barbour further assumes all the numerous remaining rings to be distributed uniformly round a circle of radius $R (\gg r)$ with centre at S , the rings being at relative rest but all having the same rotation $a\dot{\phi}_i = c$. He finds these contribute to L^2 , to the appropriate approximation, the amount

$$\{\frac{1}{2}mM(\dot{r}^2 + r^2\dot{\theta}^2 + \dot{\chi}^2 + c^2)\}/R$$

According to his postulates, the motion of the test ring is obtained by treating the sum of the two contributions as the effective Lagrangian \bar{F} , dividing for convenience by the constant M/R . For a well known property allows us to use \bar{F} instead of its square root, provided the variable t has been suitably normalised. The Lagrange equation in χ then gives

$$\partial \bar{F} / \partial \chi = \chi + B\chi = K$$

where $B = Rm_s/rM$, and K is a constant.

At this point Barbour assumes $K=c$, and he takes $B \ll 1$ as it is in his application. Then by further appeal to standard theory of variational principles, he shows an equivalent Lagrangian \tilde{F} for r, θ to be, to the required approximation

$$\tilde{F} = (\dot{r}^2/2) + (r^2\dot{\theta}^2/2) + (Rc^2m_s/Mr) + (Rm_s\dot{\phi}^2/Mr) - c^2(Rm_s/Mr)^2$$

In order to give the model a tentative

physical meaning, Barbour identifies the particular ring S with the Sun, the test-ring with a (small) planet, the distant rings with the rest of the universe. He can then identify R, M with the conventional radius and mass of the universe, and then he quotes the 'cosmic coincidence' that the Newtonian cosmological constant $G \approx Rc^2/M$, provided c be taken as the speed of light. Then in \tilde{F} the first two terms are the inertia terms and the third the gravitation term in the Lagrangian for a particle moving in the field of a mass m_s according to Newtonian mechanics and gravitation. So Barbour considers he is on the track of an explanation of inertia and gravity "in terms of the single simple concept of mass in relative motion" and that this "hinges on the existence of the conjectured internal motion." The latter he would naturally expect to be more complicated in a more realistic model. He goes on to speculate on the possibility that "the rest-mass energy of the particles is associated with an incessant internal motion at velocity c ", so that the internal motion would account for both inertial and gravitational mass. He hopes for a generalisation of his Lagrangian that could be regarded as comprehending special relativity and quantum mechanics.

The last two terms in \tilde{F} are what Barbour calls (small) Machian terms. He states that the corresponding terms in the analogous three-dimensional model yield one-sixth of the Einstein value for the advance of the perihelion of a planet. Presumably he hopes for a better value in a more developed treatment, and he mentions several other expected developments. Lastly he suggests that his treatment may be regarded as a realisation of a system of forceless dynamics envisaged by Hertz but never constructed.

It is easy to ask several questions: Can the treatment be applied to other obvious problems? What is the status of the geometry and of time in the theory? What is the justification for the use of a variational principle and for the Lagrangian employed? Has it a meaning to say that all energy may be kinetic since, if there is no rest mass, we ask what carries the kinetic energy? Also there are questions of detail: For example, why does Barbour identify his constant K , which is a constant of a particular motion, with c which appears as a universal constant in his scheme?

Nevertheless, at this stage the work is clearly exploratory and one should ask rather what promising positive indications are emerging. It is certainly so

interesting that one must wait at least to see what Barbour will do next. It might prove to be just another way of developing general relativity, or it might yield general relativity with certain constraints that render it Machian in

some sense in which general relativity is not necessarily Machian. Or, as Barbour seems chiefly to hope, it may give something like Machian relativity incorporating some sort of quantum mechanics. □

Synaptic remodelling in the mutant cerebellum

from Shin-Ho Chung

OVER the past few years, anatomical studies on neurological mutant mice have shed new light on the mechanisms governing the formation of the mammalian nervous system. In particular, the cerebellar cortex of these mutants has proved to be fertile ground for investigating certain fundamental questions about neural development. This is because the cell types and their interrelationships, along with the normal sequence of developmental processes, are better understood in the cerebellum than in most other parts of the nervous system. Because a single gene mutation often interferes with developmental processes in a precise and specific way, examination of the affected brain will reveal the primary target of the mutation as well as the consequences of such a previous mistake on the subsequent formation of the brain. A recent ultrastructural study on the 'weaver' cerebellum by Sotelo (*Brain Res.*, **94**, 19-44; 1975) is aimed at elucidating these questions.

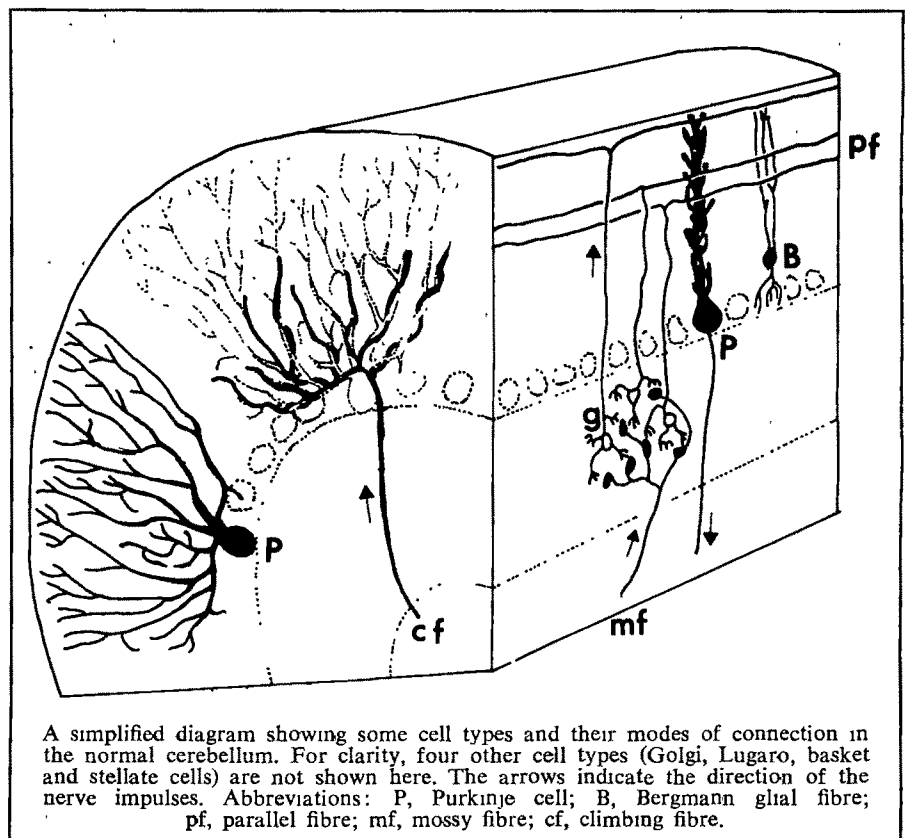
The cerebellum of the normal mouse contains six types of neurones distributed in three layers; these are, from the outside in: the molecular layer, the Purkinje cell layer and the granular layer. The cells interconnect in regular patterns and integrate the inputs conveyed from other parts of the brain by two main afferent fibre systems. The Purkinje cell bodies which lie in a single row just above the granular layer, extend their flattened dendritic trees towards the surface of the cerebellum. Climbing fibres, one of the two main inputs to the cerebellum, form numerous synaptic contacts with the shafts of these Purkinje cell dendritic trees (see figure). The second input (mossy fibres) influence the Purkinje cell mainly through granule cells. Profuse branches of the granule cell dendrites curve around and encapsulate the expanded terminal of a mossy fibre, forming a tangled ball of dendrites and axons. Axons of the granule cells, known as parallel fibres, are extended towards the molecular layer, where they bifurcate, and running perpendicular to the Purkinje cell dendrites,

form numerous synapses with their spiny branchlets.

When this intricate structure is severely perturbed, as in the homozygous weaver mouse, the animal exhibits behavioural abnormalities characterised by tremor, hypotonia and ataxia. The autosomal, recessive weaver gene mutation arose spontaneously, and subsequent histological examination of the affected animal revealed that almost the entire population of granule cells is missing (Lane, *Mouse News Lett.*, **30**, 32; 1964). During postnatal development of the normal mouse, postmitotic granule cells migrate down from the germinal layer (near the surface of the cerebellum) through the Purkinje cell layer to form the granular layer. Rezaei and Yoon (*Devl Biol.*, **29**, 17; 1972) first showed in the homozygous weaver mutant that granule cells proliferate normally, but remain at the germinal

layer and gradually die. From these observations, Rezaei and Yoon suggested that the massive cell death is a consequence of the failure to migrate.

This conclusion was supported by subsequent investigations. In a series of electron microscopic studies, Rakic and Sidman (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 240; 1973; *J. comp. Neurol.*, **152**, 103, 133; 1973) noted that the disorder is accompanied by defective Bergmann glial fibres. It had been shown previously that these glial fibres guide the migration of the granule cells into the granular layer (Rakic, *J. comp. Neurol.*, **141**, 283; 1971). Early in development, before the granule cells have divided, numerous glial cells extend their Bergmann fibres radially towards the external granular layer. The postmitotic granule cell then attaches itself to the endfeet of the glial fibre to descend to the inner region of the cerebellum, while undergoing an ordered series of transformations in shape as it migrates. As the cell body descends, it leaves a T-shaped axon behind, which is to become a parallel fibre. Thus, in order to move across a complex terrain that changes in composition with time, migrating granule cells use the Bergmann fibre as a sliding ladder. Rakic and Sidman believe that the target of the weaver gene mutation is the Bergmann glial abnormality which causes, and precedes, failure to migrate and the death of granule cells. As evidence for this assertion, they note that the glial abnormality can be detected long before the



appearance of granule cells, and that migration is slowed along the abnormal glial fibres in heterozygotes. Moreover, morphological examinations show no difference in the granule cells of normal and weaver mice before they start to migrate. Sotelo and Changeux (*Brain Res.*, **77**, 484; 1974), however, dispute this conclusion. In the homozygous animal carrying the weaver mutation but with a genetic background different from that of the mice studied by Rakic and Sidman, they find that Bergmann fibres appear almost normal. This was also found by Bignami and Dahl (*J. comp. Neurol.*, **155**, 219; 1974) who studied the development of Bergmann fibres in various neurological mutants using an immunofluorescence technique. Furthermore, the few granule cells that do migrate successfully undergo a similar course of degeneration as the postmitotic cells that fail to migrate.

Although the primary target of the genetic abnormality thus remains unsolved, its consequences on synaptogenesis have been analysed by Sotelo (1975) and Rakic and Sidman (*J. comp. Neurol.*, **152**, 103, 133; 1973). Because mutant cerebellum is agranular, the mossy fibre is deprived of its normal postsynaptic target, and apical dendrites of the Purkinje cell lack the afferent terminals which would occupy these synaptic sites. Probably because of the existence of unoccupied synaptic sites calling for innervation, there is an extensive remodelling of the cerebellar circuitry, as well as reshaping of the dendritic geometry. Instead of assuming the normal pattern of the dendritic tree with profuse tertiary branches, the Purkinje cell dendrites are oriented haphazardly and their thickened primary and secondary branches, without the usual spiny branchlets, continue to grow to the pial surface where they may turn inward. By contrast, the Purkinje cell dendrites in normal animals seem to recognise some kind of invisible barrier that excludes them from penetrating into the external granular layer. These aberrant, even inwardly oriented, dendrites are nevertheless entwined faithfully by climbing fibres, but their apical segments which are normally occupied by parallel fibres are frequently innervated by climbing fibres also. Failing to find their proper targets, mossy fibres extend their terminals and penetrate towards the superficial half of the cerebellum. The endings may then be encapsulated by dendrites of other types of neurone with which they normally do not form exclusive synaptic junctions.

These observations highlight some of the unsolved problems in neurobiology. The development of the nervous system involves a number of events

occurring in sequence. From the germinal layer, neurones migrate to their eventual destinations and extend dendritic and axonal arborisations characteristic of each cell type before functional connections are made between one neurone population and another. Since the cerebellar disarray in the weaver mouse results from the failure of granule cells to migrate, other neurones should be normal in appearance if their properties are determined solely by intrinsic epigenetic mechanisms. That the shape of Purkinje dendrites is drastically altered, and mossy and climbing axons extend their processes further than they normally do, suggests the importance of an interaction between a neurone and its milieu in the moulding of the nervous system. Moreover, the observation that synaptic contacts can be formed in the weaver cerebellum between pre- and post-synaptic elements which normally do not form junctions, shows that intrinsic specificity for afferent-efferent coupling can easily be overridden by an altered environment. Such a flexible strategy in the developmental programme is indeed one of the ways of minimising the effects of a previous mistake, genetic or epigenetic, in the subsequent formation of the nervous system. □

Thermodynamic errors by international commission

from D. R. Wilkie

ALAS, the gulf still seems wide between some branches of physiology and the base of physical chemistry on which biology must ultimately rest. Even so it is hard to understand how a distinguished international group comprising 11 physiologists (after consultation with 57 others) came to put forward recommendations that are simply at variance with the laws of thermodynamics. Even more remarkable, these recommendations have apparently remained unchallenged for the one and half years since they were first published, suggesting that confusion about this topic is indeed widespread.

The International Commission of the IUPS for Thermal Physiology has published detailed recommendations concerning the names and units to be employed by physiologists engaged in studying metabolism, thermal balance, and so on (Bligh and Johnson, *J. appl. Physiol.*, **35**, 941; 1973). This is a praiseworthy enterprise, and the Commission has made one or two radical recommendations, the most striking (page 941) being

that "In the BODY HEAT BALANCE EQUATION, the symbol M is now used to denote METABOLIC FREE ENERGY PRODUCTION; METABOLIC HEAT PRODUCTION is identified by the symbol H ". By "free energy production" is clearly meant the rate of change of free energy associated with the chemical processes of metabolism, taken with a negative sign. This new definition has much to commend it since it is the changes of free energy, rather than of energy or enthalpy, that determine the potentiality of chemical reactions to drive the processes essential to life—such as biosynthesis and thus growth, production of electrical or mechanical work, and active transport.

The proposal is somewhat idealistic since free energy changes are usually difficult to measure. There are abundant calorimetric determinations of the energy and enthalpy changes associated with the oxidation of foodstuffs, but I at least do not know of similar systematic determinations of free energy changes since the pioneering work of Burk (*Proc. R. Soc.*, **B104**, 153; 1929) who showed for the oxidation of carbohydrate that the free-energy change was almost equal to the enthalpy change. It cannot be assumed that the same near equality applies for the oxidation of other foodstuffs: and it certainly does not apply for other important metabolic reactions such as the hydrolysis of ATP. In any case, when one is concerned purely with questions of energy balance (as in most of thermal physiology), the free energy change is not directly relevant. Confusion over this point has led to the incorrect equation (recommendations page 943):

"BODY HEAT BALANCE EQUATION: A mathematical expression that describes the net rate at which a body generates and exchanges heat with its environment (First Law of Thermodynamics):

$$S = M \pm E - (\pm W) \pm R \pm C \pm K$$

[W] or [W m⁻²]

in which

S = rate of STORAGE OF BODY HEAT (+ for net gain by body)

M = METABOLIC FREE ENERGY PRODUCTION (always +)

E = EVAPORATIVE HEAT TRANSFER (− for net loss)

W = WORK (+ for POSITIVE WORK against external forces)

R = RADIANT HEAT EXCHANGE (+ for net gain)

C = CONVECTIVE HEAT TRANSFER (+ for net gain)

K = CONDUCTIVE HEAT TRANSFER (+ for net gain)."

This equation is incorrect. It is compatible with the First Law only if one substitutes for their definition of M :

" M = the total rate of change of enthalpy resulting from the chemical processes of metabolism, taken with a negative sign".

I have since confirmed in a helpful correspondence with Dr Bligh that the Commission was not fully aware of the fundamental distinction between 'free energy' and 'energy' or 'enthalpy'. Clearly it will take some time to frame modified recommendations that do take account of this difference: meanwhile the recommendation quoted from page 943 (and others derived from it on pages 949, 950, 951 and 958) should be interpreted with great caution.

Basically the relation between the chemical processes of metabolism and their physical manifestations, work produced (w) and heat produced (h), is perfectly simple, (Wilkie, *Prog. Biophys.*, 10, 260; 1960). In modern terminology we have, under strictly isothermal conditions, over a defined time interval Δt :

$$h + w = \Delta \xi_1 (-\Delta H_{m1}) + \Delta \xi_2 (-\Delta H_{m2})$$

and so on

$$= \sum_0^j \Delta \xi_i (-\Delta H_{mi}) \quad (1)$$

where the subscripts 0– j designate the various chemical processes occurring; the $\Delta \xi_i$ represent the extents by which each reaction has advanced (measured most conveniently as additional mol of a named product that have appeared); and the ΔH_{mi} are the molar enthalpy changes of the various reactions measured calorimetrically and expressed in kJ per mol of the same named product formed.

Note that if the organism is in a steady state—and the experimental design in most metabolic studies aims to achieve this—the situation becomes very simple indeed since the $\Delta \xi_i$ of all the intermediary reactions are then zero. If only a single substrate (carbohydrate for example) is being oxidised the right hand side of equation (1) then becomes reduced to a single term (Wilkie, *J. Mechanochem. Cell Mot.*, 2, 257; 1974). At first sight this may seem surprising since the intermediary reactions are still proceeding and contributing to the heat produced. That they are equivalent to a single global reaction is a consequence of Hess's Law.

Thermal physiologists are generally not interested in strictly isothermal conditions since they are concerned with what happens when the body temperature θ changes. They are also concerned with the various ways in which heat can be transferred from the body to the environment by evaporation (e), by radiation (r), by convection (c) and by conduction (k). Equation (1) can be readily adapted for this purpose. Rearranging into roughly the same form as that quoted from the recommendations:

$$C\Delta\theta = \sum_0^j \Delta \xi_i (-\Delta H_{mi}) - w - e - r - c - k \quad (2)$$

C is the thermal capacity of the body (SI unit $J^\circ C^{-1}$) so the left-hand side is the change of thermal energy, the "storage of body heat". All the terms in the above equation are quantities of energy (J); each can be converted to power (W) by dividing by Δt .

Note that when work is done on the organism rather than by it (so-called "negative work") w simply appears in Equations (1) and (2) with its sign changed.

Consequently there seems to me to be no need to introduce the distinction between "TOTAL HEAT PRODUCTION" and "METABOLIC HEAT PRODUCTION" presented in the recommendations; it merely confuses further a

situation that is already confused enough.

One final point of criticism is that I think that the Commission showed poor judgement in endorsing the use of the phrase "energy metabolism" (pages 946 and 951). It would be far safer to restrict the word "metabolism" to the chemical events in living organisms so as to maintain a clear distinction between the chemical changes and the changes of physical energy that result from them. This is not merely a matter of semantics. Confusion by physiologists over this distinction has recently provoked the misguided suggestion (Banks and Pearce, *Lancet*, i, 811; 1975) that energy changes are irrelevant in considering metabolic processes. □

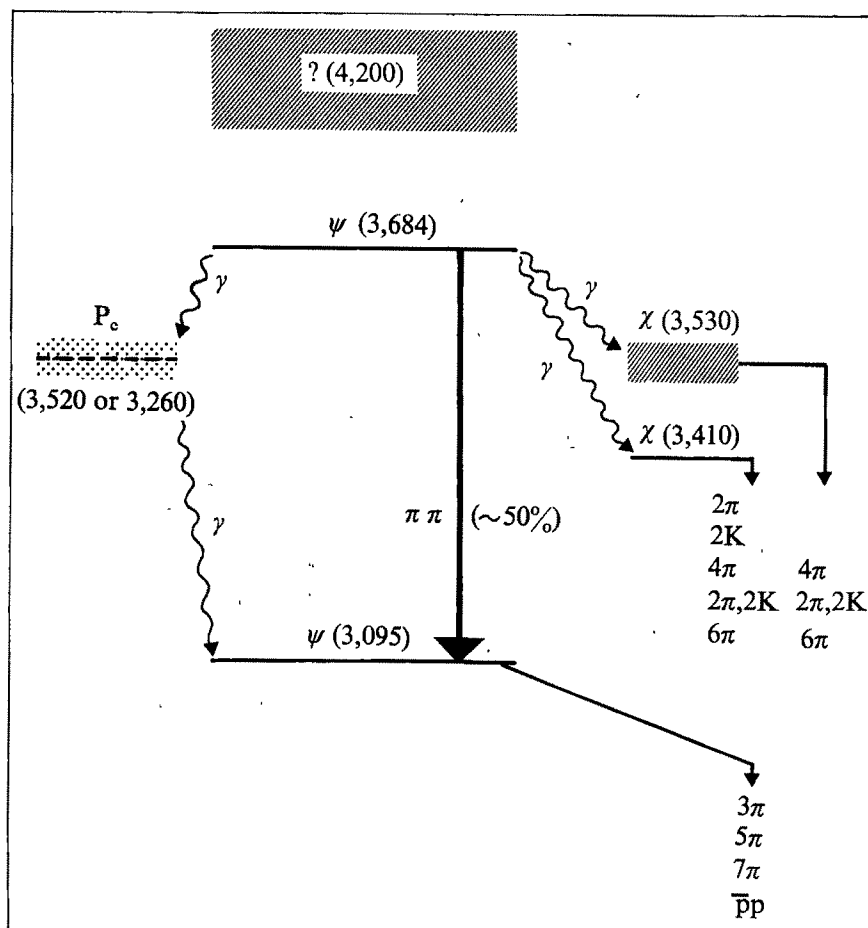
More new particles

from W. Toner

Two remarkably long-lived new particles were discovered last November: the ψ (mass 3,095 MeV), alias J; and the ψ (3,684). At least two new states related to the ψ s, have now been observed in detailed studies of the decays of the ψ

(3,684), and it begins to be possible to sketch a tentative level scheme, as shown in the figure. A preliminary report of these studies was given in *News and Views* for August 21.

The evidence for the state named P_c comes from a group working at the electron-positron storage ring DORIS, in Hamburg and is discussed in a paper by Braunschweig *et al.* (*Phys. Lett. B*,



Tentative level scheme (July/August 1975). Only a small fraction of the decays have been identified, and only a few of those are shown. The 3,095, 3,684 and 4,200 are formed directly in electron-positron annihilation. P_c may (or may not) be the same as χ (3,530).

57, 407; 1975). They have shown that a few per cent of ψ (3,684) decays lead to the formation of the ψ (3,095) by the emission of two γ rays. They also have data which strongly suggest that the decay proceeds through an intermediate state (or cluster of states) which they have called P_c . At present they do not know whether the more energetic of the two γ rays is emitted first or second, so that their mass determination is ambiguous.

The states named χ in the figure were discussed at the recent Lepton-Photon Symposium at Stanford by Feldman *et al.*, the Stanford-Berkeley collaboration working at the SPEAR ring in Stanford, California. This group discovered the ψ (3,095) (simultaneously with Ting, at Brookhaven, who called it the J) and the ψ (3,684). They observe that a few tenths of a per cent of ψ (3,684) decays proceed by the emission of a γ ray and two, four or six strongly interacting charged π and K mesons. In the decay ψ (3,684) $\rightarrow \pi^+ \pi^- \pi^+ \pi^- \gamma$, the four pions form one or other of two very distinct states χ (3,530) and χ (3,410) whereas in ψ (3,684) $\rightarrow \pi^+ \pi^- \gamma$, the two pions form only the lower state, which is narrow (that is, long-lived). Both states appear to be formed in the 6π and 2π 2K channels. The upper state is broad, and may be a group of states. It may (or may not) be the same as P_c .

The discovery of these states ends a period of uncertainty about the general nature of the mechanism which gives the ψ s their remarkably long lives. Any explanation invoking a new quantum number whether 'charm' or 'colour' or something else inevitably required there to be a complex set of states, some of which could be reached by the emission of a γ ray from a ψ . Indeed, most models predicted much greater radiative decay rates than are observed, and inspired the earlier fruitless searches at lower sensitivity which gave rise to the uncertainty. Now that they have been seen, 'charm' is once again a hot favourite, and a multitude of detailed models is being developed in which the 'obvious' decay modes are suppressed by one subtlety or another. In these models, the ψ s, χ s and P_c though made of a charm-anticharm pair of quarks have charm = 0. Overtly charmed particles with charm = ± 1 would have to be produced in pairs. Again, fruitless searches have been carried out for theoretically obvious decay channels, and the models are now being adjusted accordingly. Colour enthusiasts have not yet given up, and of course, it could be something else altogether.

The experimenters need not involve themselves in the theoretical controversy and are being careful about the precise wording of their interpretations. For example, the broad? (4,200) state shown in the sketch is not claimed as a particle

by the Stanford-Berkeley group. They describe it as an enhancement which could be one or more new particles or, possibly, an effect connected with the threshold for some other new phenomenon. They are assured that their data—and they have a great deal more in the course of analysis—will have the last word. □

The peptide hormones: molecular and cellular aspects

from D. G. Smyth

Biosynthesis and release of secretory hormones formed the subject of a Symposium arranged by the Ciba Foundation in honour of Sir Frank Young (London, June 30–July 3). The proceedings are to be published in full by Associated Scientific Publishers, Amsterdam.

It is beginning to be accepted that most if not all peptide hormones are biosynthesised as fragments of larger molecules. The search is now on for the ultimate precursors, synthesised on the ribosome and translated from messenger RNA. J. F. Habener (Massachusetts General Hospital) described the primary biosynthetic form of parathyroid hormone, the 'preparathyroid hormone', which contains a peptide of 31 amino acids attached to the N-terminus of the 84-residue hormone. The major part of the extension, comprising the first 25 amino acids, is excised shortly after biosynthesis and before transference of the 90-residue prohormone to the secretory granule. The existence of an analogous precursor to insulin, a preproinsulin, was strongly supported by the experiments of M. A. Permutt (Washington University) who isolated RNA from catfish islets and observed its ability to stimulate protein synthesis in a wheat germ cell-free system: among the proteins formed was a major component with a molecular weight of 11,000, larger than catfish proinsulin. The *in vitro* synthesised product shared antigenic determinants with catfish insulin and appeared to be identical with a rapidly labelled protein synthesised by the islet tissue. From mammalian pancreas the isolation of mRNA has proved more difficult because the islets comprise only 1% of the tissue and ribonuclease is highly active. D. F. Steiner (University of Chicago), working with a microsomal fraction from rat islets, concluded that the signal peptide must be detached from the preprohormone on the ribosome before the proinsulin

chain is completed. He suggested there may be two distinct enzyme-catalysed steps in the export of secretory hormones, the pre-processing stage which does not involve a trypsin-like enzyme and the pro-processing stage which does. The conversion of some prohormones to hormones, however, may involve enzymes with specificities different from trypsin, and activation may take place not by hydrolysis but by aminolysis of the peptide chain (D. G. Smyth, NIMR, Mill Hill). This transamidation reaction would account for the origin of the C-terminal amide that is a characteristic feature of many of the peptide hormones and both the known releasing factors. On this basis it would be predicted that LHRF, TRF, gastrin, oxytocin, vasopressin, secretin, α -MSH, and others, will be found to arise from prohormones or pro-releasing factors that extend from the C-terminal residues.

β -MSH

The fragments formed by enzymic activation of a prohormone are retained in the secretory particle of the gland. From pig pituitary, β -MSH and two novel peptides were isolated in equivalent amounts; the three peptides represented contiguous fragments of the 91-residue polypeptide termed β -LPH and appear to have been released by an enzymic mechanism similar to that involved in the activation of proinsulin (A. F. Bradbury, D. G. Smyth and C. R. Snell, NIMR). On this evidence, it was proposed that β -LPH is the prohormone of the lipolytic hormone β -MSH. Final proof of the relationship between β -MSH and β -LPH will be obtained by isolation of the prohormone from the secretory granule and study of its conversion by granular enzymes. It is of interest that β -LPH and its 58-residue fragment γ -LPH, which are only weakly active as lipolytic agents, are present in substantial quantity in pig pituitary and this raises the possibility that the enzymic release of the hormone from these precursors may be under physiological control. Lesley Rees (St Bartholomew's Hospital) reported that β -MSH could not be detected by immunoassay of human pituitary extracts and maintained that in the human the physiological significance of this hormone is in question.

ACTH

Precursors to several hormones have been indicated by immunoassay of glandular extracts or plasma (Rosalyn Yalow, Veterans Administration Hospital, Bronx). The high sensitivity of the method allows analyses to be performed on serum from a single individual but inevitably the conclusions drawn are limited to assessments of

molecular size and convertibility of the products. A 'BIG' ACTH was found in tissue extracts of carcinoma primary to or metastatic from the lung. Excluded from Sephadex G50 and with low corticotrophic activity, 'BIG' ACTH was converted by controlled tryptic digestion to a product with high activity, apparently identical in size and charge to (1-39)-ACTH. Curiously, an intermediate size ACTH seems to be present in rabbit, rat and mouse whereas the usual 39-residue peptide predominates in other species. Yalow suggested that the hormonal form of ACTH may be a factor in regulating the cortisol:cortisone ratio in mammalian corticoid secretion. Working with pig pituitary, Bradbury *et al.* (NIMR) isolated and sequenced a new 38-residue peptide which was present in large amounts; they considered that it may be part or all of the N-terminal region of a prohormone to ACTH.

Gastrin

A 'BIG' gastrin, predicted by S. A. Berson and Rosalyn Yalow, has now been isolated from antral mucosa or

Zollinger-Ellison tumour tissue (R. A. Gregory, Liverpool University) and its sequence determined. In this precursor, a heptadecapeptide extends from the N-terminus of the 17-residue hormone and, as with the prohormones of insulin, glucagon, PTH and β -MSH, it is clear that activation is by enzymic cleavage at the C-terminal side of paired basic residues. The list of gastrins and gastrin precursors has grown to include component 1 (Cl), 'big big' gastrin (BBG), 'big' gastrin (BG, 34 residues), little gastrin (LG, 17 residues) and minigastrin (G, 13 residues); all these are duplicated by corresponding forms with sulphated tyrosine. In addition, it is known that the C-terminal pentapeptide retains full activity.

Hormone activity regulation

P. J. Randle (University of Bristol) suggested that the regulation of hormone activity may take place not by control of the rate of enzymic attack on a prohormone but by alteration of specificity. Covalent modification of certain enzymes by phosphorylation,

which is sensitive to calcium, could lead to the formation of prohormone fragments that differ in size, potency, and possibly in duration of action. In general, the conversion of prohormones to hormones is being found to take place by way of numerous intermediates, some stable some transient. Only when these have been isolated in homogeneous form and subjected to biological scrutiny will the ground be cleared for understanding the physiological role of each component.

In the closing presentation, J. R. Tata (NIMR) called attention to two classes of response initiated by peptide hormones. Rapid responses involve change in permeability in the membranes of target cells, directly affecting the levels of intracellular intermediates; slower effects take place through stimulation of RNA and protein synthesis. It seems likely that different classes of hormone receptor are concerned. Tata proposed that the ultimate expression of growth and maturation results from the cooperative interaction of the two types of response. □

DESPITE the availability of undisturbed sites, the arctic tundra has, until recently, been one of the most understudied of the world's terrestrial biomes. Even now there are many leading questions concerning general ecosystem function and balance within this biome which remain unanswered. Studies of primary production by arctic ecosystems, both in the field and in the laboratory, have been receiving some attention recently, for example by Larry Tieszen at Barrow, Alaska (*Arctic and Alpine Res.*, 4, 307; 1972, and 5, 239; 1973) and results have now been published of a carbon dioxide flux study which has been undertaken at the same locality by Coyne and Kelley (*J. appl. Ecol.*, 12, 587; 1975).

The study of carbon dioxide flux between soil, vegetation and the atmosphere has certain useful attributes as a method of approaching gross ecosystem energetics. In particular it avoids the problems of extrapolating from small samples studied in detail, either by cropping, or by CO_2 exchange studies in enclosed conditions, or by $^{14}\text{CO}_2$ incorporation studies. The analysis of data obtained is difficult, however, because of both the meteorological and the biological variables involved. In effect, a flux of CO_2 from ground to atmosphere means that respiration of producers, consumers and decomposers exceeds photosynthetic consumption at that point

in time. The reverse is true if CO_2 flux is from atmosphere to vegetation.

Coyne and Kelley worked through an arctic growing season (mid-June until the end of August) and regularly took air samples at five heights

Carbon dioxide flux in arctic ecosystems

from Peter D. Moore

up to 16 m, which they analysed for CO_2 concentration using infrared gas analysis. They also monitored various micrometeorological variables, such as wind speed, light and air temperature. For much of this season, the ecosystem received light for a full 24 h (diurnal variation, about 50 W m^{-2} at night to 250 W m^{-2} at mid-day). Diurnal air temperatures varied from 4 to 24°C . CO_2 flux also varied diurnally; on July 21, midnight values were about $-0.2 \text{ g m}^{-2} \text{ h}^{-1}$ (that is, flux from ground to atmosphere), and at mid-day, about $+0.6 \text{ g m}^{-2} \text{ h}^{-1}$. For about 17 h of the day in late July there was a positive flux of CO_2 from the atmosphere to the vegetation. For the remaining 7 h, photosynthesis failed to consume all of the CO_2 produced by ecosystem respiration. In late June and early August, the negative flux periods

were of 12-14 h duration per day. In these early and late season times, there was a daily net flux of about $-1 \text{ g CO}_2 \text{ m}^{-2}$. In the height of the season daily net flux was $+8 \text{ g CO}_2 \text{ m}^{-2}$.

Over the entire season (61 d), it was calculated that $626 \text{ g CO}_2 \text{ m}^{-2}$ was generated by ecosystem respiration. This would largely be due to the primary producers and the decomposers, the latter also responding to raised temperatures by increased activity. During the season $146 \text{ g CO}_2 \text{ m}^{-2}$ (net) was consumed by primary producers in photosynthesis; thus gross production would amount to $772 \text{ g CO}_2 \text{ m}^{-2}$. If one applies the crude conversion factor from CO_2 incorporated to dry weight accumulated of 0.614, then this represents 474 g m^{-2} dry matter (gross) in a season. The net production figures compare reasonably with Tieszen's values obtained by more conventional methods, which lends credibility to the data derived from these flux studies.

This new approach has thus provided further information concerning the consumption and generation of CO_2 in the tundra ecosystem. If the ecosystem is in a stable, equilibrium state, then one must assume that the net seasonal gain of $146 \text{ g CO}_2 \text{ m}^{-2}$ is either being returned to the atmosphere by off-season decomposition or is accumulating as litter or peat within the system.

Interstellar dustmen's convention

from Ant. Whitworth

A workshop on Interstellar Grains was organised by University College Cardiff at Gregynog Hall on July 11-13.

DUST gets into everything, or so they always say, and certainly it seems the cosmos is no exception. In astrophysics, dust grains (sub-micron sized particles constituted of compounds of Si, C, N, O, Fe, Mg) are continually invoked, and invested with new capabilities, to explain puzzling observations. Yet the recent increase in constraints on dust models has not resulted in a more definite picture of dust grains, but rather in the introduction of more free parameters (such as multi-component models and localised variations). Nevertheless, a pattern emerges, and with it the realisation that grains have many very fundamental roles in the cosmos.

At the recent workshop H. C. van de Hulst (Sterrewacht, Leiden) stressed that the interstellar medium is a very hostile environment for grains; and J. M. Greenberg (Sterrewacht, Leiden) pointed out the need to consider how intense irradiation may modify grain properties. J. P. Bibring (Laboratoire René Bernas, Orsay) has investigated grains in lunar and meteoritic samples, and concluded that irradiation of grains in circumstellar environments—say by stellar winds, energetic photons, or cosmic rays during their formation—may significantly round the grain and amorphise its internal structure. D. R. Huffman (University of Arizona, Tucson) pointed out that this bears on the identification of the $10\text{ }\mu\text{m}$ feature in infrared spectra which requires amorphous silicates; whereas the $2,200\text{ }\text{\AA}$ ultraviolet feature requires well-ordered graphite. S. Ramadurai (Institute of Astronomy, Cambridge) argued that carbonaceous chondrites contain interstellar graphite grains from the protosolar nebula, as evidenced by their anomalously high boron content, which is attributed to cosmic ray bombardment of graphitic carbon.

But a cogent case was made by N. C. Wickramasinghe (University College, Cardiff) for considering grain materials with a volatility intermediate between the very volatile 'dirty ices' (hydrides of C, N and O), and the more refractory silicates and graphite. He cited organic polymers, and in particular suggested that conditions in dark clouds may favour condensation of the widespread formaldehyde mole-

cule into elongated grains of polyoxymethylene (POM). There remains outstanding the problem of producing sufficient formaldehyde, but there is already good evidence in support of the general thesis. D. A. Mendis (University of California, La Jolla) reported on the physical parameters for the grains producing 10 and $18\text{ }\mu\text{m}$ features in comets, and indicating an evaporation temperature $\sim 500\text{ K}$: this rules out silicates, but is very compatible with many polymers, including POM. B. Thomas and co-workers (University of Wales Institute of Science and Technology, Cardiff) have measured strong absorption bands around 10 and $18\text{ }\mu\text{m}$ in thin films of POM.

M. J. Barlow (Sussex University) proposed a resolution of the problem of supplying sufficient refractory grains to the interstellar medium: refractory grains condense in almost every stellar mass loss situation, and are probably only destroyed by direct involvement in star formation after a mean lifetime $\gtrsim 10^9\text{ yr}$. D. P. Gilra (University College, Cardiff) deduced the existence of a temperature minimum in the atmospheres of late N-type carbon stars where SiC_2 grains are condensing. C. D. Andriess (Sterrewacht, Roden) presented infrared observations of η Car as evidence of dust suddenly condensing in the mass outflow at a radius $\sim 0.03\text{ pc}$. H. Okuda (Kyoto University) interpreted polarisation data on VY Can Maj in terms of a circumstellar dust disk: is this disk protoplanetary? J. Silk (University of California, Berkeley) prescribed a scenario for fragmenting and reconstituting dust in cocoons around newly formed massive stars.

W. W. Duley (York University, Ontario) offered new hope for identifying the diffuse bands: he attributed narrow lines to impurities in the bulk grain material, and the adjacent broader features to the same impurities in grains which are so small that their lattice constant is altered. D. P. Gilra (University College, Cardiff) calculated infrared absorption cross sections for small ellipsoidal particles, taking account of surface modes, and stressed the great importance of grain shape. A. P. Whitworth (University College, Cardiff) argued that in neutral clouds dielectric grains are charged stochastically on a time scale $\sim 10^3\text{ s}$, typically having 0, ± 1 electron charges. Consequently, grains are effectively untied from the magnetic field, can move through the gas under gravity and/or radiation pressure, and can be aligned in the required sense. P. A. Aannestad (University of Arizona, Tucson) suggested that stochastic grain charging may also enable grains to deplete C^+ , N^0 and O^0 equally, as inferred from observation. But D. A. Williams (Uni-

versity of Manchester Institute of Science and Technology) concluded that the depletion of CNO may anyway be negligible.

A number of interesting roles have been suggested for grains in cosmology. A. J. R. Prentice (University of Oxford) argued that in the rotating protosolar cloud, grains fall to the centre creating a core sufficiently massive to stabilise the rest of the cloud, and establishing composition gradients which will explain the solar neutrino deficiency. I. P. Williams (Queen Mary College, London) has shown the basic part which grains may play in forming the planets and determining their different compositions. M. Rowan-Robinson (Queen Mary College, London) poses the question: is dust always associated with violent events in galactic nuclei? One might also ask whether such events are not a major source of the dust in the cosmos. \square

Galaxy formation

from Janet E. Jones

A workshop meeting on "The Formation and Evolution of Galaxies" was held at the Institute of Astronomy, Cambridge, on August 4-8, and supported by the Gravity Research Foundation.

LEAVING aside the still controversial topic of the origin of pre-recombination density inhomogeneities, the emphasis at the workshop was focused on later epochs, with theories for early galactic evolution and observational "clues" on the topic being well discussed. The workshop opened with a number of papers on the covariance and multiplicity functions, the purpose being to use the presently observed spatial distribution and luminosity functions of galaxies as indicators of the primordial density fluctuation spectrum.

It was pointed out that non-linearities would tend to increase the ultimate slope of the covariance function, thus making it more difficult for an initially flat, $n=0$ (Poisson), density fluctuation spectrum to account for the observed covariance function: an $n=-1$ initial spectrum seems to give a better fit (J. R. Gott and M. Rees, Institute of Astronomy, Cambridge). Moreover, the slope of the covariance function seems to depend on galaxy type, being steeper for ellipticals than for spirals (M. J. Geller, Harvard University). This could suggest some difference in the primordial density fluctuation spectrum leading to the two types, although later attempts to model galactic evolution did not incorporate this property. In-

deed, a notable non-feature of the workshop was the absence of any attempt to propose a genuine bifurcation scheme for elliptical versus disk-like galaxies. P. Schechter (Institute for Advanced Study, Princeton), W. Press (Princeton University), Gott and Geller all discussed aspects of the multiplicity and luminosity functions for galaxies. The multiplicity function for the Gott-Turner groups of galaxies seems to be consistent with an initial Poisson density fluctuation spectrum (Schechter), although, as with the covariance function, an initial $n = -1$ spectrum gives a slightly better fit (Gott).

B. Jones (IOA, Cambridge) reviewed turbulence theory for the origin of primordial density perturbations, and discussed the effect of the recombination process on the covariance function derived from both the cosmic turbulence theory and the adiabatic density perturbation theory. This, and a later more specialised discussion by S. Bonometto (University of Padua), emphasised some of the difficulties inherent in both these theories for the origin of primordial density inhomogeneities.

The discussion then moved on to the topic of galactic evolution. It was argued that galaxies evidently originate from a single protogalactic cloud, since the globular clusters display a range of properties that vary systematically through the Galaxy, and from one galaxy to another (S. van den Bergh, University of Toronto). But this "monolithic" unit may have increased in size as a result of infalling material, as suggested by measurements of stellar metallicities in the solar neighbourhood (D. Lynden-Bell, IOA, Cambridge). Direct calculation of the collapse of a turbulent protogalactic cloud led to fragmentation into $10^{5-7} M_{\odot}$ clouds, with the formation of a massive central star in each cloud fragment, inhibiting further star formation (B. Jones, and S. Weber, University of California, Berkeley). It is not clear how further evolution would proceed from this point. At any rate, the two theories of galactic evolution presented at the workshop each included an assumed star formation rate without regard to the detailed physical processes involved. The first model by Gott and T. X. Thuan (CalTech) took as its starting point a rotating protogalactic cloud, and assumed that star formation occurred over some characteristic timescale, T_{st} . If this were short compared with the collapse timescale of the galaxy, T_{tr} , then the result was an elliptical system, and if long ($T_{st} > T_{tr}$) it formed a disk system. The second model (R. Larson, Yale University) began with a turbulent protogalactic gas cloud, in which star formation took

place at an assigned rate during collapse. If the cloud had low angular momentum an elliptical system was formed, and if high angular momentum, a disk system formed, though some additional adjustment of other parameters was required in the latter case.

Neither model is perfect. Evidently, elliptical systems are easier to make than spiral ones, though even here there were some notable discrepancies.

Gott's model being dissipationless produced a majority of EO systems though it had the nice property that the maximum flatness produced was E7 (Gott and Thuan). Observations suggest however that E3 is the most common type. Conversely Larson's model tended to produce too much flattening. In each case, flattening was said to have derived from rotation.

But both models gave the observed luminosity profile for elliptical galaxies, assuming a constant mass to light ratio. Various studies suggest, however, that the mass to light ratio may vary; it is early days yet for the galaxy builders.

The situation as regards the formation of spiral galaxies is even less satisfactory. There seems to be some difficulty in producing a clear division of type between ellipticals and spirals when starting with the same initial conditions and continuously varying parameters. Van den Bergh pointed out that SOs were not truly intermediate in type between the two classes, their ellipticity and luminosity profiles both being characteristic of spirals. He emphasised the value of the bulge/disk ratio as an indicator of spiral type and it was even suggested (Ostriker and Faber) that all galaxies could be classified by means of their elliptical component, going from large ellipticals, through spirals, to dwarf elliptical systems. But the mechanism by which the disk is produced is not clear.

Faber suggested that the bulge/disk ratio may be a factor in the removal of gas, arguing for the presence of a galactic wind in spheroidal systems; however, van den Bergh pointed out that this could not be the only factor involved in gas loss, since ellipticals and SOs are predominantly found in clusters. He therefore supported the idea that gas is stripped from galaxies by the ram pressure of an intergalactic gas. This process was proposed by G. R. Gisler (IOA, Cambridge) to explain the very low gas content found in most elliptical systems, where population synthesis studies indicate that more gas is being lost by stars than is evidently consumed by new star formation (B. Tinsley, Yale University, and W. O'Connell, University of Virginia). This assumes a normal stellar mass function, since there is no obvious way to manufacture only low mass stars.

But since the ram pressure effect could not easily account for the variations in gas content in spiral systems it seems likely that more than one process is operating to influence the gas content of galaxies in general.

The last problem to be considered was the possible detection of galaxies in the process of formation. An argument based on free-fall times suggested that galaxies would have formed between a redshift of 50 and 5, and perhaps as recently as $z=3$ (Larson). An elegant study by D. Meier (University of Texas, Austin) based on the models of Larson and Tinsley, predicted a possible spectrum for a newly formed galaxy, and indicated that its nucleus could be visible as a QSO. He proposed two candidate objects worthy of further investigation in this field: OH471 and 4C0534. The principal argument against Meier's work is that it neglects the effect of dust. This is a significant omission, since if most of the radiation were absorbed by dust and re-emitted in the infrared, it may be difficult or impossible to detect (M. Rowan-Robinson, Queen Mary College, London). But what is particularly unfortunate for those engaged in the task of galaxy making is that there are apparently no reasonable candidates for galaxies in the process of formation now (W. L. W. Sargent, CalTech). Presumably, this implies that either there are no more suitable protogalactic clouds around, or that conditions are no longer right for turning them into galaxies, or both. In the words of Sidney van den Bergh: "Galaxies are like people: they depend on both genetics and environment". This, indeed, was the theme of the workshop. □



A hundred years ago

A UNIVERSITY is to be founded at Tomsk, one of the chief towns of Siberia. The new establishment will have only two faculties, one of Law and the other of Medicine. The want of doctors in Siberia may be inferred from the fact that there are only fifty-five of them in a country which is as large as the whole of Europe, and whose population amounts to more than 6,000,000 inhabitants. The Russian Minister of Finance has granted a credit of 40,000*l.* on the revenue of the State for the new establishment, which will raise the number of Russian Universities to eight.

from *Nature*, 12, 446; September 16, 1875.

review article

Unified gauge theories of elementary particles

E. Leader & P. G. Williams*

Recent theories which unite weak and electromagnetic interactions provide new hope for a unified theory of fields and their interactions. Experimental developments such as the discovery of neutral currents and the new psi particles lend encouraging support for some of the ideas embodied in these theories.

One of the principal goals of 20th century physics has been to obtain a unified understanding of all the basic interactions observed in nature. Einstein spent his later years attempting to unify electromagnetic and gravitational interactions. His failure, and that of some of his contemporaries, was one of the reasons why the pursuit of such grand theoretical syntheses lost its popularity. Instead the main effort of fundamental physics has been concentrated on less ambitious, more limited goals, studying each interaction more or less in isolation.

In the past three years unification has once more come into vogue through some quite remarkable theoretical and experimental developments in high energy physics. In this article we shall attempt to outline the ideas behind these developments.

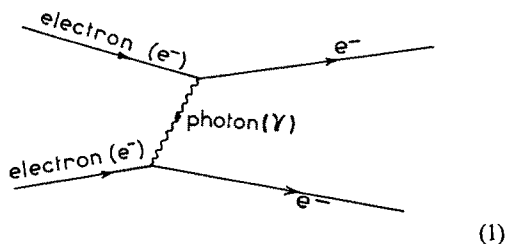
The past 40 years have led to the elucidation of four distinct types of forces or interactions acting between elementary particles: the strong, the electromagnetic, the weak and the gravitational forces, with relative strengths in the ratios $1 : 1/137 : 10^{-13} : 10^{-39}$. Whereas gravity acts upon all particles the other forces seem to be more selective. Table 1 lists a few of the known elementary particles and the forces they experience.

When hadrons interact it is difficult to study their weak and electromagnetic properties since their strong interactions are predominant. We cannot switch off the strong interactions; but nature has provided its own probe—the leptons—which seem to have no strong interactions. For the moment we shall confine our attention to the leptons, returning to hadron physics again later.

Electromagnetic interaction of leptons

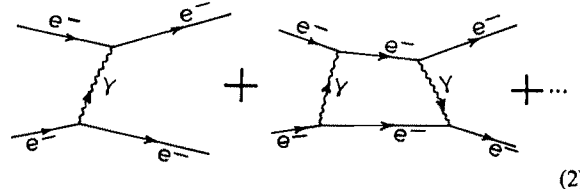
A typical leptonic electromagnetic process is the scattering of one electron by another: $e^- + e^- \rightarrow e^- + e^-$. Classically the two negatively charged electrons repel each other and are deflected from their original paths.

In quantum field theory the interaction is pictured as arising from the exchange of an electromagnetic wave (that is a photon) between the two electrons as in (1).

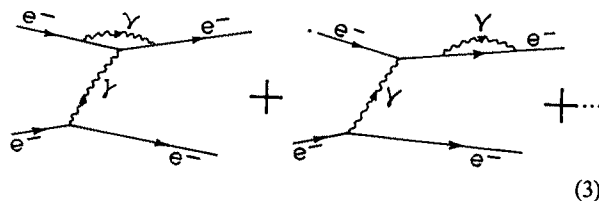


But this is not the only mechanism contributing to the scattering: two or more, in fact any number of photons can be

exchanged. The physical process is described by the sum total of all such mechanisms (2).



Each of these "Feynman" diagrams corresponds to a precise mathematical expression. Each photon exchanged represents one interaction with the electromagnetic forces. Fortunately these forces are so weak that the more photons there are in a diagram the smaller its contribution. The force produced by the exchange of a single photon depends on the strength of the "coupling" between the photon and the electron. This is equal to the electric charge, e , of the electron. In Fig. 1 the force is proportional to $e^2 = 1/137$. Thus the scattering process is described by a series of successively decreasing terms proportional to e^2 , e^4 , e^6 , and so on. Unfortunately some diagrams give rise to mathematical expressions which when evaluated are infinitely large. An example occurs at order e^4 where each of the three diagrams in (3) is infinite—but miraculously their sum is finite! Crucial to this cancellation of infinities is



the "universality" of the coupling of the photon to electrically charged matter: the fact that the force is always proportional to the electric charge, and is always the same; the electric charge is "conserved". Quantum electrodynamics is riddled with these infinities, but they always cancel. A field theory with this remarkable property is said to be "renormalisable".

It is sometimes said that the finiteness of the theory depends on the fact that the photon has zero mass; but this is not the case. One can imagine a theory of electrodynamics with massive photons and this is also renormalisable. That the mass of the photon is zero is an experimental fact intimately connected with the inverse square law, and known to great accuracy. A consequence of the zero mass of the photon is that electromagnetic waves are transverse, having only two possible polarisation states, both perpendicular to the direction of propagation.

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Table 1 Properties and interactions of some elementary particles. Superscripts denote the electric charge in units of the electronic charge e : thus μ^+ has charge $+e$; μ^- charge $-e$

Generic name	Interactions	Particles	Antiparticles
Hadrons	Strong Electromagnetic and Weak	Proton p	Antiproton \bar{p}
		Neutron n	Antineutron \bar{n}
		Pions π^+	π^-
		π^0	π^0
Strange particles		Kaons K^+	K^-
		K^0	\bar{K}^0
		Lambda Λ^0	Antilambda $\bar{\Lambda}^0$
Leptons	Weak and electromagnetic	Electron e^-	Positron e^+
		Muon μ^-	Antimuon μ^+
	Weak	Electron	Electron
		Neutrino ν_e	Antineutrino $\bar{\nu}_e$
		Muon	Muon
		Neutrino ν_μ	Antineutrino $\bar{\nu}_\mu$

A massive "photon" could also have a longitudinal polarisation and therefore a total of three polarisation states. These states of longitudinal polarisation are additional sources of infinities when the "photon" has a mass, but they cancel as a result of charge conservation. That is why the electrodynamics of massive "photons" is also renormalisable.

To summarise: the quantum electrodynamics of electrons and photons is a complete theory in the sense that all physical processes involving only electrons and photons can be calculated to arbitrary accuracy by perturbation theory, and the results are finite. The experimental record of quantum electrodynamics is impeccable: there are no known deviations from the theory and some predictions agree with experiments to within parts per million.

We have remarked that the cancellation of infinities in quantum electrodynamics is miraculous. In the next section we shall examine the mechanism behind this miracle.

Gauge invariance

To every conservation law in physics there corresponds a symmetry of the dynamical laws. For example, energy and momentum conservation are consequences of the symmetry of physical laws under translations in time and space: angular momentum conservation is a result of symmetry under rotations.

Charge conservation, the root cause of the renormalisability of quantum electrodynamics, is connected with the fact that the electromagnetic potentials are not unique, but may be altered in a particular way at different points in space and time without any change in the form of the laws of electrodynamics. In a quantum field theory these changes in the electromagnetic potentials are always accompanied by a change in the fields that represent the particles; the particles react to the effect of the change in the electromagnetic potentials, but in just such a way as to leave the physics unaltered. This symmetry is termed gauge invariance. To emphasise that each point in space-time has its own different gauge transformation we use the nomenclature "local" gauge invariance.

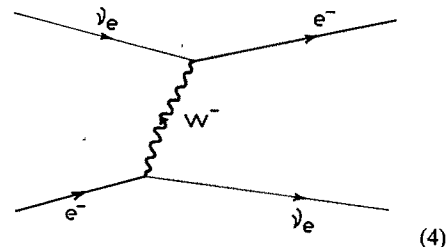
Not only does local gauge invariance lead to charge conservation, but it also forces the photon mass to be zero. But as we mentioned before massive photons also give a renormalisable electrodynamics, so that local gauge invariance is a larger symmetry than needed for charge conservation and for renormalisability. It turns out that all we require for charge conservation is a "global" gauge invariance where the gauge transformations are the same at all space-time points. The photon may have a mass without violating this symmetry.

This approach to charge conservation may seem rather formal, but it lends itself to generalisations that will lead to a theory for the weak interactions.

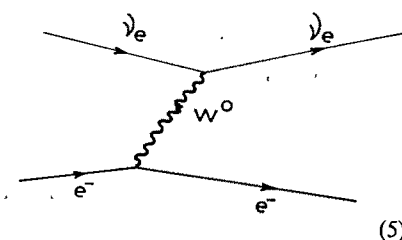
Weak interactions

At first sight it might seem that weak interactions have no features in common with electromagnetic processes. The β decay of the neutron into a proton, electron and antineutrino ($n \rightarrow p + e^- + \bar{\nu}_e$), the decay of muons $\mu^- \rightarrow e^- + \bar{\nu}_e + \nu_\mu$, are very different from typical electromagnetic interactions. Besides being far weaker, they violate parity conservation (that is left-right symmetry) and involve very short range forces; electromagnetic forces conserve parity and are long range as a result of the zero mass of photons. Yet Fermi, who first wrote down a theory of weak interactions in a remarkable paper in 1934, based his ideas on a close analogy with electrodynamics.

Let us imagine doing an experiment analogous to electron-electron scattering considered earlier, but involving only weak interactions. A good example involving only leptons is the elastic scattering of neutrinos off electrons, $\nu_e + e^- \rightarrow \nu_e + e^-$. By analogy with electrodynamics we shall assume that the weak force responsible for this scattering process is caused by the exchange of some "weak photon" called the W meson or W boson, as shown in (4). Note from the figure that since the neutrino has no electric charge, the W boson must have the same charge as the electron. As will be discussed later, until



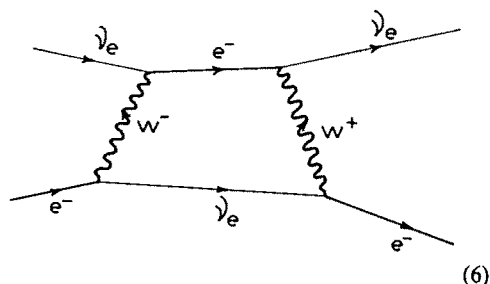
recently all experiments suggested that only charged W boson exchange occurred. If there were a neutral W boson, we could have also drawn a second diagram (5).



The first diagram (4) is called a "charged current" interaction; the second (5) a "neutral current" interaction. The latter bears the closest relation to electromagnetism because the photon also has no charge.

Now it can be shown that the range of the force generated by the exchange of a particle is inversely proportional to its mass. Experiment shows that the weak interactions are so short ranged that the W boson mass must be greater than that of any known particle. (Fermi's original theory corresponds to an infinite mass W.) Our failure so far to observe any W bosons could well be because our accelerators are not energetic enough to create them in the laboratory. Their discovery would constitute by far the most decisive evidence in favour of the picture we have painted of weak interactions.

The theory outlined above—both in Fermi's original form and with a sufficiently large W mass—has been most successful in correlating the various phenomena of weak interactions. But it only works in the lowest order of perturbation theory. In our example of neutrino-electron scattering, the next most complicated term to be added to (4) is shown in (6) and is infinite.

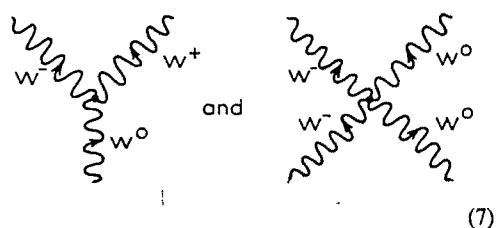


(6)

Unlike quantum electrodynamics there is no way of cancelling the infinities of the theory—it is non-renormalisable. The trouble lies in a failure to pursue the analogue with electrodynamics closely enough: the theory contains a "weak photon" (the W boson) but there is no "weak charge" conservation, and the experience of quantum electrodynamics suggests that this is the ingredient needed to obtain a renormalisable theory. This problem was solved by Yang and Mills in 1954.

Yang-Mills gauge theories

The difficulty of constructing a gauge invariant theory of the W bosons is that unlike the photon they must have electric charge. The photon, which only interacts directly with charged particles will not interact directly with itself. But Yang and Mills showed that because W bosons are charged they must interact with themselves and to achieve a gauge invariant theory one must also have a neutral W^0 in addition to W^+ and W^- . The possible diagrams are much more complicated now because three or four Ws can interact with each other as shown in (7).

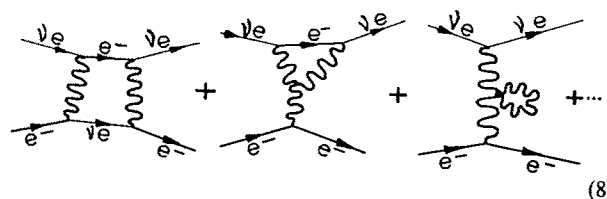


(7)

These Yang-Mills theories are a natural extension of electrodynamics, because they possess the full local or space-time dependent gauge invariance only when the W bosons are massless, but the introduction of masses still preserves the conservation of a "charge". This is, of course, not the electric charge but something we shall wish to identify as the weak charge alluded to in the previous section. We have already mentioned that electrodynamics with massive photons is renormalisable because charge conservation is sufficient to guarantee this without need for the full local gauge invariance.

Weak interactions are short range and therefore require massive W bosons. The analogy with electrodynamics with massive photons suggests that a massive Yang-Mills theory will be renormalisable and, therefore, could provide the basis for a theory of weak interactions.

At first it might seem that massive Yang-Mills theories are indeed renormalisable. Thus, in the example of elastic neutrino-electron scattering the introduction of a self-interacting W boson leads to the addition of further diagrams to the infinite two W boson exchange diagram (8).



(8)

All these diagrams are individually infinite, but their sum is finite—the infinities cancel out exactly. Unfortunately it was later shown that such cancellations do not always occur and therefore massive Yang-Mills theories are not, in fact, renormalisable. It turns out that the full local gauge invariance is a necessary requirement and this means that the W bosons cannot be given a mass, although experiment requires it.

The inescapable conclusion seemed to be that gauge theories, except in the simplest case, electrodynamics, do not apply to the real world. In 1971, however, a Dutch graduate student, 't Hooft, opened the flood-gates by giving convincing arguments for the renormalisability of a rather special type of Yang-Mills gauge theory. Such theories circumvent the difficulties described in this section by an ingenious and elegant device.

Secret symmetries and Goldstone bosons

To describe the developments that led to the salvation of gauge theories we must digress to consider the concept of a "secret symmetry", sometimes confusingly called a "spontaneously broken symmetry". This idea concerns physical systems displaying properties which seem to violate the symmetry possessed by the underlying physical laws. The best example of a secret symmetry is a permanent bar magnet. The interactions between the atoms and molecules are certainly invariant under rotations yet the bar magnet is magnetised in one particular direction. The magnet is not invariant under rotations, because the direction of magnetisation is singled out as a special one, but the underlying physical laws are. What application does this have to high energy physics? Perhaps the W bosons having a non-zero mass are like the bar magnet. Perhaps the underlying physical laws are invariant under local gauge transformations, despite the non-zero mass of the W's.

In 1961, Goldstone pointed out that any system having a secret symmetry pays a price for its secrecy: there must exist zero frequency modes or, in the case of relativistic field theory, there must exist spinless zero mass particles. These particles are called Goldstone bosons. In the ferromagnet, Goldstone bosons correspond to spin waves propagating through the solid. This would seem to spell the end of hopes for using secret symmetries in gauge theories because no zero mass Goldstone bosons have ever been observed in high energy physics experiments.

In 1964, Higgs, and independently Brout and Englert made a discovery, the true significance of which has only recently been fully appreciated. The Goldstone theorem has buried in it the assumption that there must be no long range forces; otherwise the chain of reasoning leading to zero mass Goldstone bosons breaks down. Of course, zero mass vector mesons such as the photon and the W bosons of Yang-Mills gauge theories do produce long range forces and so these theories avoid the strictures of Goldstone's theorem—Goldstone bosons need not exist.

Higgs showed that not only do zero mass Goldstone bosons not need to occur, but that a gauge theory with secret symmetry

can be made to use a spinless particle—now called the Higgs meson—to give the W bosons a mass. The freedom supplied by gauge invariance enables us to 'mix-up' the Higgs meson with a third polarisation state of the W bosons. In other words, the zero mass gauge particles which possess two states of polarisation, and the Higgs meson, which possesses one state of polarisation, combine to produce massive Ws with three states of polarisation, and the Higgs meson as an independent particle disappears. In the process the gauge symmetry seems to have been lost, but it is still there only rather well hidden. Although this fact was understood at the time, it was felt that since the symmetry is so well and truly hidden by the Higgs mechanism, there was no reason for the theory to be renormalisable.

Unified gauge theories

In 1967 two short papers appeared in which the electromagnetic and weak interactions of leptons were unified in a gauge theory using the Higgs mechanism. One by Weinberg suggested that the theory might be renormalisable; the other by Salam argued that since the underlying theory has a gauge symmetry, perhaps the Higgs mechanism does not spoil its renormalisability.

The Salam–Weinberg theory barely caused a flutter until 1971 when 't Hooft gave his non-rigorous but convincing arguments that theories of this type are indeed renormalisable. 't Hooft's work caused a considerable stir because it then became possible for a gauge theory to apply to weak interactions. Since an essential ingredient in a wide class of gauge theories is the existence of an electrically neutral W boson, extensive experimental work was initiated, not to find it directly, but to see its effects as an exchanged particle producing a weak force—as a neutral current.

Before we discuss the experimental evidence for neutral currents let us briefly describe the main features of the Salam–Weinberg theory. First we should stress that the theory originally concerned only the weak and electromagnetic interactions of leptons, the electron e^- and its neutrino ν_e , the muon μ^- and its neutrino ν_μ . The Salam–Weinberg theory is a gauge theory with a total of four gauge vector mesons all starting out with zero mass; but the Higgs mesons in the theory are carefully chosen to give a mass to only three of the bosons when the symmetry becomes a secret one. These are the W^+ , W^- and W^0 of the weak interactions. The fourth gauge boson remains massless and is identified with the photon. The combined existence of all four of these particles is a necessary part of the gauge symmetry itself and therefore of the renormalisability. There are ways of avoiding a neutral W boson; but the price to be paid is heavy: the number of leptons must be increased by the introduction of heavy leptons.

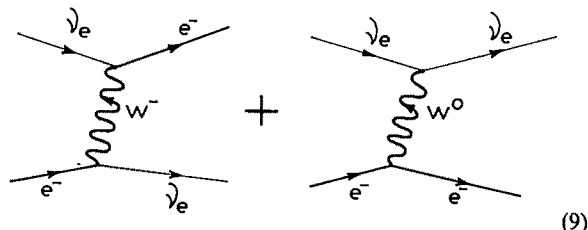
In what sense does such a gauge theory represent a unification of electromagnetic and weak interactions? This comes about through the special status that the gauge particles have in a gauge theory—they all couple to matter and to each other with the same universal coupling strength $e^2 \approx 1/137$. But whereas the exchange of zero mass particles (photons) produces an effective coupling e^2 typical of electromagnetic interactions, the exchange of W bosons of mass M_w turns out to have an effective coupling e^2/M_w^2 . For this to be as small as the observed weak interaction strength requires a very large mass M_w of at least 40 GeV, or 40 times the mass of the proton! This rather general property of unified gauge theories makes it unlikely that W bosons will be produced directly in experiments in the near future. Instead experiments have concentrated on the search for neutral current effects in neutrino scattering processes.

Evidence for leptonic neutral currents

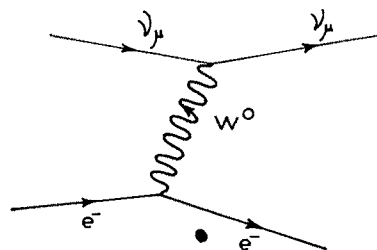
Until recently neutrino physics has been something of a poor relation in high energy physics. It is difficult to produce intense neutrino beams, and interactions are so rare that experiments take a long time to perform. In the past few years, however, with the prospect of new high energy accelerators, much effort

has been invested in new equipment, just in time to make an effective search for neutral currents.

There are only two basic processes involving leptons able to provide experimental tests for the existence of neutral currents: the scattering of either electron or muon neutrinos and anti-neutrinos off electrons. Both reactions occur at very low rates and are therefore very difficult to measure, even by the standards of neutrino physics. The first (9) would occur even in the absence of neutral currents, but the second (10) is a pure neutral current process that would be absent if only charged currents occur.

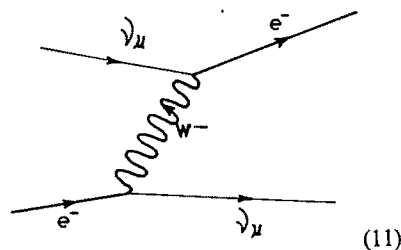


(9)



(10)

It is essential to note that (10) represents the only independent one W boson exchange diagram contributing to $\nu_\mu + e^- \rightarrow \nu_\mu + e^-$ because experiments have shown that muons and their neutrinos cannot turn into electrons and their neutrinos. These "lepton number" conservation laws are an important ingredient in the interpretation of neutral current experiments. Thus diagram (11) which would be the analogue of the first diagram in (10), is forbidden by the conservation of lepton number.



(11)

The muon neutrino experiment is the most decisive one, and was carried out at CERN using a large bubble chamber facility specifically designed for neutrino experiments. The first results were announced in 1973, showing one event believed to be $\bar{\nu}_\mu + e^- \rightarrow \bar{\nu}_\mu + e^-$. This experiment has continued to accumulate statistics, and at present the total number of neutral current events is three. It is impossible to exaggerate the difficulty of such an enterprise. Everything hinges on being certain that what is observed is not some other process. The problem is that the only observed product of the collision process is the final electron coming out of the reaction. Neither the incoming nor the outgoing neutrinos can be observed, and the electron might well result from processes involving other unobserved neutral particles such as neutrons or photons. Much of the effort and nearly all the discussion revolves around the estimation of the background events. In this experiment they have been estimated to be around 10%, but with only three events at hand who can be certain?

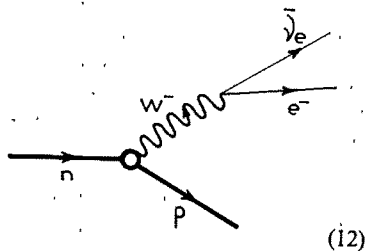
Potentially this experiment is the best test of the Salam–Weinberg gauge theory; but the most reliable evidence for neutral currents has come from neutrinos scattering off hadrons where the event rates are considerably higher. Now, however, the

strong interactions enter into the picture, to obscure and complicate the theoretical interpretation. We must consider the possible ways of extending unified gauge theories to hadrons.

Hadrons and quarks

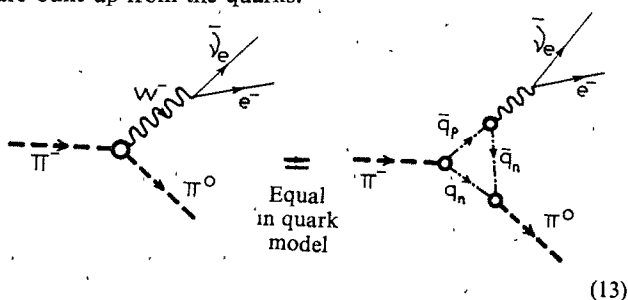
Up to now we have been discussing the leptonic world consisting of the electron, muon and their neutrinos. As far as we know the forces between these particles are only weak and electromagnetic. Indeed, the neutrinos, being neutral, do not even have electromagnetic interactions. This is why they are able to penetrate enormous quantities of material, such as stars and the Earth itself.

Besides the leptons there is a vast array of particles including protons, neutrons, nuclei, π mesons, K mesons, strange particles and many other so-called "elementary" particles all of which have strong or nuclear interactions. These hadrons have interactions amongst themselves some 100 times stronger than the electromagnetic interactions and some 10^{13} times stronger than the weak interactions. The structure and behaviour of hadrons is almost entirely determined by the strong interactions. Hadrons, however, also have electromagnetic and weak interactions, although these are heavily influenced by the strong interactions. An example of a hadronic weak interaction is β -decay of the neutron: $n \rightarrow p e^- \bar{\nu}_e$. How can we picture this interaction as the exchange of an intermediate vector boson? The answer, already implicit in Fermi's original theory of 1934 is that the neutron emits a charged vector boson which then decays into the leptons.



The coupling of the W boson to hadrons, however, is not as simple as it is to the leptons, because it is influenced by the strong interactions. The latter determine the structure and constitution of the hadrons, which it has become vogue to describe in terms of hypothetical fundamental constituents, the "quarks". In the quark model the hadrons are all built out of three quarks q_p, q_n, q_λ and their antiparticles $\bar{q}_p, \bar{q}_n, \bar{q}_\lambda$. This leads in a simple way to the famous SU(3) classification of particles. For our present purposes all we need note is that mesons such as the π or K are presumed to be bound states of a quark and antiquark, whereas baryons such as protons and neutrons are bound states of three quarks. Quarks have never been produced in experiments, but the remarkable success of the quark model is taken as evidence that it contains at least some element of truth.

The quark model simplifies the problem of weak decays of hadrons by enabling us to express them all in terms of the decays of only the quarks themselves. A simple example is the decay $\pi^- \rightarrow \pi^0 + e^- + \bar{\nu}_e$ now depicted as in (13), where the quarks are assumed to have simple weak and electromagnetic interactions like the leptons. The complications of the strong interactions are then hidden in the fashion in which the hadrons are built up from the quarks.

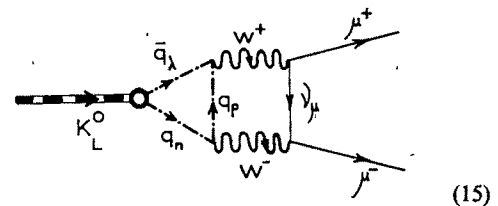
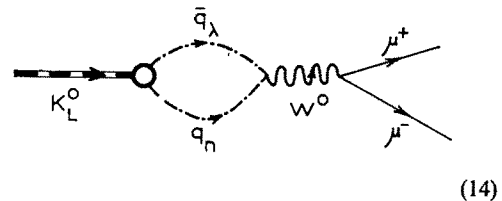


The simple symmetry properties of quarks enable them to be included in a natural elegant way in a unified gauge theory of weak, electromagnetic and possibly even strong interactions. Nearly all such theories have neutral W bosons as an essential ingredient, so that once again their exchange as neutral currents provides the best available experimental test. We shall see later that the possible existence of a new quantum number "charm" may provide even more decisive evidence in favour of unified gauge theories.

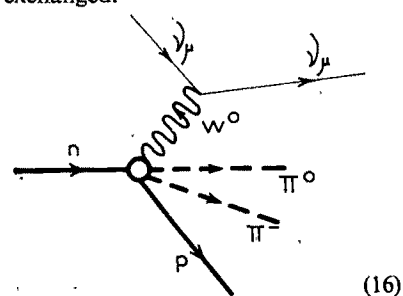
Tests for neutral hadronic currents

We have already emphasised the problematic nature of hadronic interactions; but from the point of view of gauge theories our catalogue of difficulties is by no means complete. For some years there has been powerful evidence against the existence of neutral currents in "strangeness changing" weak processes. These are processes where the initial and final hadrons have different values of the strangeness quantum number. A classic example is the strangeness changing decay $K^+ \rightarrow \pi^0 + e^+ + \nu_e$ which has been observed experimentally and which involves a charged current; but the very closely related decay $K^+ \rightarrow \pi^+ + e^+ + \nu_e$ which would involve a neutral current is suppressed by a factor of about a million.

An even more important example of a strangeness changing decay process is that of the long lived neutral K meson. This process $K_L^0 \rightarrow \mu^+ + \mu^-$ involving a neutral current is 10 million times less frequent than the similar but charged current decay $K^+ \rightarrow \mu^+ + \nu_\mu$. This suppression is so strong that not only must the lowest order diagram in (14) vanish, but the second order diagram in (15) also must be suppressed.



Before the advent of unified gauge theories it was assumed that this type of suppression occurred for all weak processes, whether or not there is a change of strangeness, and that neutral currents simply did not occur. Only with the renaissance of gauge theories resulting from belief in their renormalisability was it felt necessary to perform the difficult experiments required to check this suppression in hadronic processes not involving changes of strangeness. The first evidence was found in the summer of 1973 at CERN. Since that time further experiments both at CERN and in the USA have confirmed the existence of neutral currents that do not change strangeness. A typical event observed at CERN is $\nu_\mu + n \rightarrow \nu_\mu + p + \pi^- + \pi^0$. Since no strange hadrons are involved in the reaction there is no change of strangeness, but as (16) shows, there is a neutral W boson exchanged.

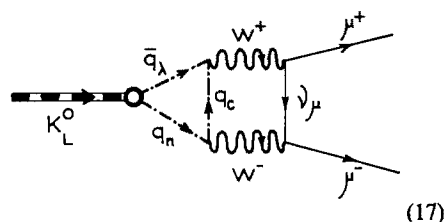


Other successful experiments have been performed which together provide conclusive evidence that neutral currents exist which do not change strangeness; but strangeness changing currents either do not exist or, if they do, are highly suppressed. What mechanism is responsible for this sharp distinction? The most elegant solution has been proposed by Glashow, Iliopoulos and Maiani.

Charm

Whenever there is a strong suppression of some otherwise innocuous process there is a good chance that a symmetry or conservation law is responsible. One way to get rid of neutral currents that change strangeness is to invoke greater symmetry so as to arrange for a universal cancellation of the unwanted processes. New symmetry means new conserved quantum numbers and new particles to carry these quantum numbers. The new quantum number invoked is "charm" and has analogous properties to those of strangeness. Just as we already have a strange quark q_s , we now need a fourth "charmed" quark q_c . It must be incorporated into a gauge theory in such a way as to preserve the gauge symmetry and therefore the vital renormalisability of the theory. In this way the unwanted process $K^+ \rightarrow \pi^+ + e^+ + e^-$ is made impossible. The unwanted $K_L^0 \rightarrow \mu^+ \mu^-$ process is, however, more subtle. The most direct way of picturing the decay shown in (14) does indeed vanish because now the W^0 cannot couple to a q_s and turn it into a q_s . But the second order diagram in Fig. 15 is still not zero.

There is now, however, an additional similar diagram involving a charmed quark in (17),



Note presence of charmed quark

which exactly cancels the diagram in (15) because of the close connection between the couplings: due to the gauge symmetry the coupling of the charmed quark q_c to W^+ and W^- is just the opposite to the proton quark q_p .

If this explanation of the absence of strangeness changing neutral currents is to be anything more than a theoretical exercise it must lead to other consequences open to experimental test. The merit of the charm theory is that it has many distinctive predictions. It invokes a new quantum number and therefore new particles carrying charm. In August 1974 M. K. Gaillard, B. W. Lee and J. L. Rosner showed how the existence of the charm quantum number would lead to an extension of the well known $SU(3)$ classification scheme to $SU(4)$ where the now familiar octets become groups of 15 or more particles. Most of the new particles have charm, but some do not. In particular the ϕ meson, a known and rather peculiar vector meson of mass 1.02 GeV or 1.08 times that of the proton, gains a partner, the ϕ_c whose estimated mass is about 2-3 GeV.

The new particles

In what sense is the well known ϕ meson peculiar? It has an unexpectedly long lifetime some twenty times greater than other unstable hadrons. Indeed, the strong decay into three light π mesons, $\phi \rightarrow \pi^+ + \pi^- + \pi^0$ which was expected to be its major decay mode is some $4\frac{1}{2}$ times less frequent than the decay into two heavy K mesons, $\phi \rightarrow K + \bar{K}$. The conventional explanation for the unusually long lifetime of the ϕ meson is based on the quark model. Although in principle ϕ can consist of any mixture of $q_s \bar{q}_s$, $q_p \bar{q}_p$ and $q_n \bar{q}_n$ quarks, in fact it is almost entirely made up of the strange quarks $q_s \bar{q}_s$. Pions are made up of protons and neutron quarks only, so that the ϕ meson finds it hard to turn into pions; but since ϕ is predominantly made of

strange quarks it has no difficulty manufacturing K mesons since these also contain strange quarks. The only problem for the ϕ is that the K mesons are so heavy that there is only just enough energy available to form two Ks. Thus the ϕ meson is long lived for two reasons: it is mainly composed of strange quarks, and strange K mesons are much heavier than non-strange π mesons.

The ϕ_c is a companion to the ϕ in the sense that it is expected to be mainly made of charmed quarks $q_c \bar{q}_c$ just as the ϕ is mainly composed of strange quarks. But the interesting possibility noted by Gaillard, Lee and Rosner was that the analogues of the strange K mesons, the charmed D mesons might be too heavy to be possible decay products of the ϕ_c . Thus the ϕ_c would find it difficult to decay at all and its lifetime might be even longer than that of the ϕ .

History would doubtless have consigned charm and all papers about charmed particles to that voluminous file entitled "unsuccessful higher symmetry schemes", had it not been for the discovery in November 1974 of a particle of mass 3.1 GeV and a lifetime 40 times longer than that of the ϕ ! This particle, currently named ψ , was seen both in hadron collision, at Brookhaven and in electron-positron collisions at the intersecting storage rings SPEAR in Stanford. It has been identified as a vector meson with many of the characteristics expected of the ϕ_c . But whether it is indeed the ϕ_c awaits further experimental work. Particularly interesting is the question of the existence of particles with charm, such as the D mesons. A slightly heavier long lived particle the ψ' , having a mass of 3.7 GeV was discovered soon after the ψ . The relationship between the ψ and the ψ' is playing an important part in elucidating the true nature of these new particles, as is the discovery in the same experiments of a much shorter lived particle of mass 4.1 GeV, the ψ'' . This proliferation of new particles has caused a great deal of excitement and activity. Speculations, explanations, confirmations, further facts and even candidates for charmed particles appear weekly. Sometimes the omens are good for charm: the ψ is the ϕ_c ; sometimes there is alarm as expected features are not found. At the moment of writing there are worries because no charmed decay products of the ψ'' have been seen; but until the dust settles there is no definite conclusion one can draw except that charm is still perhaps the most favoured explanation. The decisive test for charm is undoubtedly the observation of charmed particles such as the D mesons.

Unified theory of strong interactions?

At this point we have, in principle at least, a unified gauge theory of the electromagnetic and weak interactions of all elementary particles. Can we now take the ultimate step of including also the strong interactions? The problem with theories of strong interactions is that one cannot calculate their consequences accurately, since perturbation methods do not work, and so one can never test the starting hypotheses in detail.

Recently, however, there has been an important theoretical development using so called "renormalisation group" techniques, which has proved of value in the theory of phase transitions in solid state physics, and which may be of help in solving problems involving strong interactions. It has been possible to demonstrate that some types of interaction develop simpler and simpler properties as the energy increases, leading ultimately to a behaviour somewhat like that of non-interacting or free particles! Such theories are called "asymptotically free field theories", and it is possible within them to do some calculations based on perturbation methods.

Until the advent of unified gauge theories there were no known examples of asymptotically free field theories. What is quite remarkable is the discovery that a wide class of gauge theories possess this property. It may, therefore, be possible to incorporate the strong interactions into what would then be a truly unified gauge theory, and to make detailed predictions

which could then be tested experimentally. Much effort will no doubt be expended on this challenge in the next few years.

The present state of theory

We have now followed the development of gauge theories to the present day. Starting from the successful theory of quantum electrodynamics we have traced its extension into the realm of weak interactions. This led to Yang-Mills gauge theories with massive W bosons. The theories are renormalisable only because they possess an underlying symmetry which has been hidden but not destroyed by the Higgs mechanism. The most accessible experimental consequence of these theoretical ideas is the existence of neutral currents induced by neutral W boson exchange, and these have been detected in neutrino experiments. The most direct evidence for unified gauge theories would be the discovery of the W^+ , W^- and W^0 mesons; but their large predicted masses makes this an unlikely possibility for the foreseeable future. This large mass is the price paid for unifying weak and electromagnetic interactions.

The extension of these ideas to the weak and electromagnetic interactions of hadrons produced an immediate conflict with the

experimental fact that strangeness changing hadron decay processes such as $K_L^0 \rightarrow \mu^+ + \mu^-$ are highly suppressed. To achieve such suppression a new quantum number, charm, has been invoked, leading to a rich harvest of experimental predictions. The new ψ particles may indeed be indirect manifestations of this quantum number, but the final acceptance of the idea of charm depends on the success or failure of the experimental search for D mesons and other charmed particles.

The theory invoking charm is by no means unique, and failure to observe charmed particles will lead to its rapid demise. Success on the other hand will enable us to take a large step forward, providing circumstantial evidence in one fell swoop for a wide range of theoretical ideas. The stage may then be set for a grand unification of weak, electromagnetic and strong interactions. Whatever the ultimate fate of the theoretical ideas presented in this brief survey, there is no doubt that they have stimulated a remarkable series of experimental and theoretical developments. There is surely no more apposite comment than that of Einstein and Infeld some 50 years ago:

"To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks a real advance in science."

articles

Structure of the compact radio component in 3C236

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The compact component in the giant radio galaxy 3C236 has been studied at 0.4, 2.7 and 8.1 GHz, with resolution of up to 0.13". The overall structure is double and asymmetric. The extent is 0.8" in a position angle of $121 \pm 4^\circ$ which, within the uncertainties, is the same as that of the large outer 39' double. No difference between the spectra of the subcomponents was detected. On a scale of 0.2" there is fine structure. The radio subcomponents are almost certainly not confined and are thus the results of a relatively recent event in the nucleus of 3C236. Our results are compatible with continuous flow models which are well collimated along an axis whose alignment does not change significantly during the lifetime of the source.

SMALL radio components with relatively flat spectra are now known to occur in the nuclei of many radio galaxies and QSOs. They almost certainly have an important role in producing and maintaining the extended double radio structure and their study is crucial to any understanding of the physical processes involved.

One of the most interesting examples of a double radio source with a compact central component is the giant radio galaxy 3C236. It is the only case where the radio core is sufficiently extended that its structure can be studied in detail. In 1972 Wilkinson^{1,2}, using the radio link interferometer at

Jodrell Bank, showed that the nuclear component of 3C236 (then believed to comprise the whole source) is extended by $\sim 1''$ in position angle (p.a.) 119° . Willis *et al.*³ showed that 3C236 has an overall extent of about 39' (~ 5.7 Mpc)⁴ in p.a. 122.5° . (Throughout this article a value of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ is assumed for the Hubble Constant, implying a distance of 554 Mpc to 3C236.) The remarkable alignment of the $1''$ compact component with the 39' outer double has important implications for radio source models and deserves further investigation. We present here the results of a detailed study of the structure of the compact component of 3C236 at frequencies of 0.408, 2.695 and 8.085 GHz using several interferometer baselines out to a length of 950,000 wavelengths.

Observations

The 2.695 and 8.085 GHz observations were carried out with the NRAO four-element interferometer. This array consists of three steerable 85-foot antennae with a maximum baseline length of 2.7 km, and a 45-foot antenna located about 35 km south-west of the other elements. The 45-foot antenna is connected to the control building using a phase-stable radio link⁴ and three baselines of 33.1, 33.9 and 35.3 km are obtained by correlating the signals of the 45-foot element with each 85-foot antenna. The original three-element system has been described by Hogg *et al.*⁵ and Coe⁶. The noise after 15 min of observations corresponds to ~ 0.03 Jy at 2.695 GHz and ~ 0.05 Jy at 8.085 GHz. The source was observed on December 26 and 28, 1974, between sidereal times 04 h 30 min and

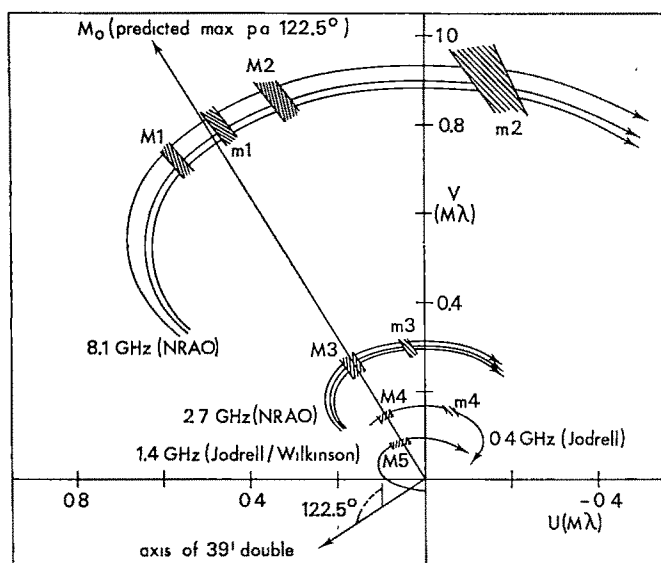


Fig. 1 The range of projected baselines used in this study. u is the east-west and v the north-south baseline in millions of wavelengths. u is measured to the left. The line denoted by M_0 indicates the amplitude maximum that would be observed from a source elongated in p.a. 122.5° (same as that of the overall $39'$ structure). M_1 , M_2 , M_3 and M_4 indicate observed fringe amplitude maxima and m_1 , m_2 and m_3 observed minima (see Fig. 2).

15 h 20 min. To calibrate the phase and gain drift, we alternated observations every 10 min between 3C236 and DA267, a nearby calibrator. The redundancy in the two days of observations helped us to estimate the residual phase and gain errors in the calibrated data. The baseline parameters of the array were obtained to an accuracy of $0.02''$ from subsequent observations of sources with accurately known positions (C. M. Wade and K. Johnson, unpublished). The visibility amplitude and phase of 3C236 were measured with respect to DA267 with an assumed position (epoch 1950.0) of $\alpha = 09^h 23^m 55.320^s$, $\delta = 39^\circ 15' 23.55''$ and assumed flux density (epoch 1975.0) of $S_{2695} = 4.5$ Jy and $S_{8085} = 9.7$ Jy (L. Blankenship, unpublished). The residual phase and gain errors were $\pm 10^\circ$ and $\pm 10\%$ at 2,695 MHz and $\pm 30^\circ$ and $\pm 20\%$ at 8,085 MHz and their effect was generally larger than that of the receiver noise.

The 0.408 GHz observations were made with a single baseline interferometer of length 122 km whose elements were the Mark Ia telescope at Jodrell Bank and one of the 25-m paraboloids at the Royal Radar Establishment, Defford. The system was basically similar to that described by Rowson⁷ with the addition of a digital delay line designed by B. Anderson. Here only fringe amplitudes were measured and no useful information was available about the absolute phase of the fringes.

The r.m.s. noise after a 1-min integration was equivalent to ~ 0.06 Jy. 3C236 was observed for a total of 12 h on July 19, 1974, and the system was calibrated using 3C286 (assumed unresolved and having a flux density of 22 Jy). Three simultaneous delay channels were used. One was centred on the compact source and gave fringes throughout the observing period. The others were optimised for the two 'hot spots' at the extremities of the $39'$ source. According to Westerbork measurements at the lower resolutions, the flux densities of these bright regions at 610 MHz are 1.8 ± 0.2 Jy and 2.1 ± 0.2 Jy respectively. We found no fringes from the hot spots larger in amplitude than 0.10 Jy, indicating that most of their flux is emitted by a region larger than $1''$.

The baseline coverage at all three frequencies is plotted in Fig. 1 together with that obtained by Wilkinson^{1,2} at 1.423 GHz. The fringe amplitude data are shown in Fig. 2.

Derivation of the radio structure

Radio maps were made at both 2.695 and 8.085 GHz by Fourier transform of the visibility data and the subsequent use of the cleaning algorithm⁸, with Gaussian restoring beams of half-power widths $0.55''$ by $0.25''$ and $0.18''$ by $0.08''$, respectively, extended in p.a. 110° . Since we know *a priori* that the compact component is smaller than $1''$ and since the NRAO array has good amplitude and phase stability, the clean procedure can be validly used here. To help judge the reliability of the cleaned maps and to interpret the data at 1.4 and 0.4 GHz where only fringe amplitude data were obtained, we fitted the fringe amplitude data at all four frequencies to various models composed of elliptical Gaussian components.

The general features of the compact component in 3C236 are displayed in the 2.7 GHz map shown in Fig. 3a. The map shows two subcomponents of unequal intensity and size. The centres are separated by $\sim 0.8''$ in p.a. $\sim 120^\circ$. The easterly component is the stronger and is not highly resolved whereas the westerly component is extended with roughly the same orientation as the outer double. In Fig. 1 the line through the origin denoted by M_0 indicates the projected baselines at which the central visibility maximum should occur for a source elongated in (and symmetrical about) p.a. 122.5° . The positions of the fringe maxima and minima observed at 2.7, 1.4 and 0.4 GHz all agree with those expected from the separation and position angles defined in the map. The parameters of the best fitting double model are given in Table 1 and the fringe amplitude function predicted by this model is shown by the solid curve in Fig. 2b. This extent and position angle agree well with

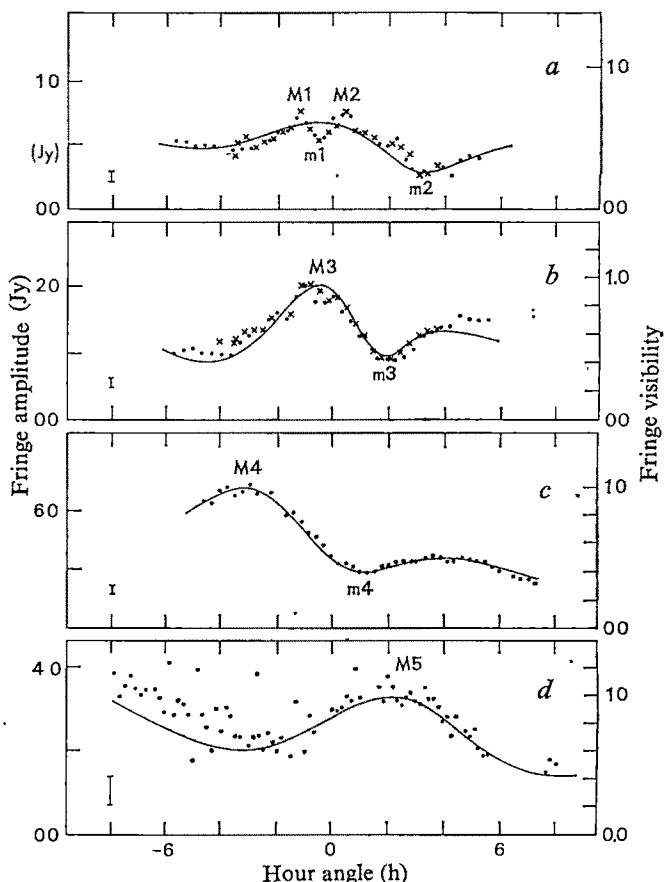


Fig. 2 Variation of fringe amplitude with hour angle at: a, 8.1 GHz; b, 2.7 GHz; c, 0.4 GHz; d, 1.4 GHz. For 8.1 and 2.7 GHz, only data from the longest of three almost equivalent baselines are shown. Bars indicate estimated total uncertainties (including systematic errors) in each fringe amplitude point. Solid lines show the fringe amplitude variations predicted by the models given in Table 1 (Fig. 2b, c, d) and Table 2 (Fig. 2a) \bullet , December 26; \times , December 28.

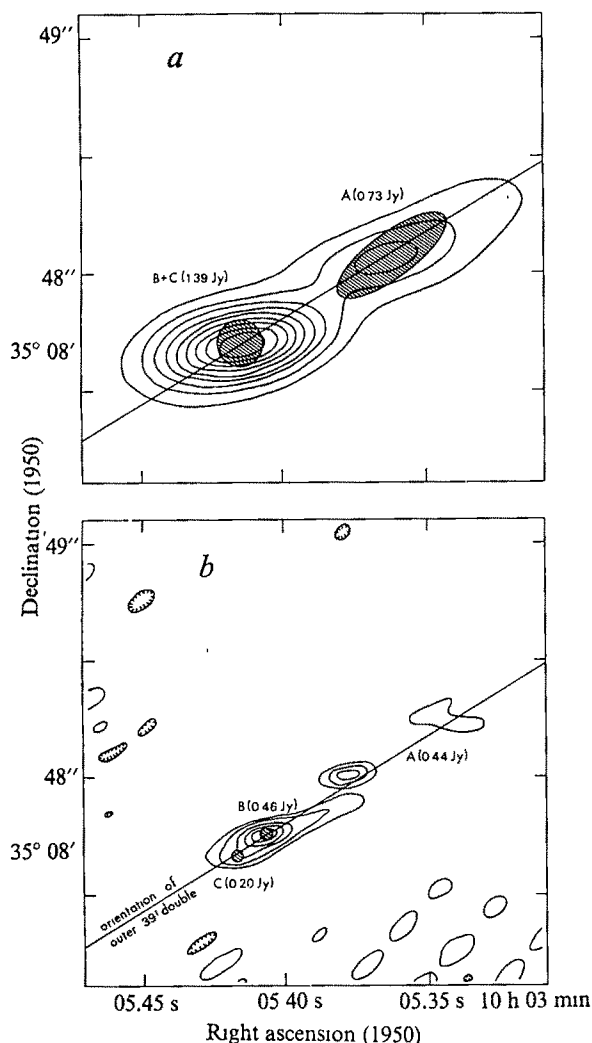


Fig. 3 Cleaned contour maps of the compact component at: *a*, 2.7 GHz with 10% contour levels; *b*, 8.1 GHz with 20% contour levels; and an additional level at 10%. The light lines at a position angle of 122.5° indicate the orientation of the 39' extended radio structure. The model brightness distribution given in Tables 1 and 2 are indicated by the shaded regions. The absolute positional accuracy is better than $0.05''$.

those previously derived from measurements at lower resolution^{1,9}.

Our observations at 0.4 GHz and those by Wilkinson at 1.4 GHz have smaller resolution than the measurements at 2.7 GHz. The fringe amplitude data are consistent with the double picture deduced from the data at 2.7 GHz. Taking the optimum 2.7-GHz model and changing only the flux densities of the subcomponents results in reasonable agreement with the observations at 0.4 and 1.4 GHz (see Fig. 2*c* and 2*d* and Table 1). There is no indication that the subcomponents have appreciably different spectra between 0.4 and 2.7 GHz.

In the case of the measurements at 8.1 GHz, the resolution is a factor of three higher and the interpretation of the structure is more complex. The easterly subcomponent dominates and is itself resolved into at least two parts denoted by B and C, separated by $0.16''$ in p.a. $121 \pm 10^\circ$. This is also apparent from the deep fringe amplitude minimum m2 and the subsequent rise in the 8.1-GHz data. The optimum parameters corres-

ponding to a point double model are shown in Table 2 and the corresponding fringe amplitude variation is plotted in Fig. 2*a*. The fit is good.

Both the map and fringe data show that the westerly component A is highly resolved. Two relatively weak hot spots are present on the map at low level but are somewhat uncertain. The effect of the westerly subcomponent in the fringe amplitudes can be seen as a weak beating near transit, corresponding to the maxima M1 and M2 and the minimum m1. This beating occurs only during a limited hour angle range because of the elongated nature of this subcomponent. The line joining the origin with the central maxima M3 and M4 in Fig. 1 intersects the 8.1-GHz baseline ellipses at a position corresponding to the fringe minimum m1. Therefore, the central maximum cannot be represented as a straight line in the u - v plane. This indicates that the structure is not completely linear. The brightest regions of the $0.8''$ source define a line which is slightly rotated with respect to that of the overall emission.

We can summarise our main conclusions (see Table 3) regarding the structure of the compact source in 3C236 as follows:

- (1) The overall brightness distribution displays a double but asymmetric characteristic.
- (2) The source extent is $\sim 0.8''$ (2 kpc).
- (3) The overall position angle is $121^\circ \pm 4^\circ$.
- (4) The easterly (brighter) subcomponent is resolved into two parts, B and C, each smaller than $0.05''$ (< 130 pc) and separated by $\sim 0.16''$ in p.a. $121^\circ \pm 10^\circ$.
- (5) The westerly component A is significantly larger, elongated by $\sim 0.6''$ (1.6 kpc) along the source axis. Perpendicular to this axis the extent is $\sim 0.1''$ (0.3 kpc). There is some indication of fine structure.
- (6) On a scale of $< 0.2''$ there is structure which is not symmetrical about the overall source axis. This asymmetry limits the accuracy of conclusion (3).
- (7) Between 0.4 and 2.7 GHz there is no appreciable difference between the spectra of A and that of (B+C).

Comparison with structure of outer radio double and optical galaxy

The 39' double structure in 3C236 has a well collimated appearance with bright frontal edges or hot spots. The two outer components have roughly equal flux densities at 610 MHz, but the westerly component is less highly collimated than the easterly one and its frontal edge is well resolved at 610 MHz with the $57'' \times 99''$ beam of the Westerbork telescope. With respect to the compact nuclear component the outer edges are located at $+24.1'$ in p.a. $122.7 \pm 0.5^\circ$ (east) and at $-15.5'$ in p.a. $121 \pm 2^\circ$ (west).

Note the following similarities between the structure of the 39' double and that of the compact nuclear component. First, the overall position angles agree to within 2° , better than the uncertainties. Second, the transverse size of the westerly structure is larger in both cases. At the position of subcomponent B the angle subtended by the westerly subcomponent A is $\sim 8^\circ$, roughly equal to the angle subtended by the outer westerly component. The radio source is identified¹¹ with a 16 mag elliptical galaxy having a redshift of 0.098. The extent of the galaxy is about $10''$ but the absolute positional accuracy of the available optical photographs is insufficient for a comparison with the radio map in Fig. 3. The galaxy is oriented in p.a. $30 \pm 5^\circ$ (A. Bridle, personal communication). Within the

Table 1 Double elliptical Gaussian model derived from 2.7-GHz data

Subcomponent		Half intensity diameters		Position angle	Separation	Position angle	Flux ratios		
		Major	Minor				2.7 GHz	1.4 GHz	0.4 GHz
West	A	$0.57''$	$< 0.20''$	$126^\circ \pm 9^\circ$	$0.77 \pm 0.04''$	$121 \pm 4^\circ$	1.9 ± 0.2	1.8 ± 0.2	1.69 ± 0.15
East	B+C	$< 0.20''$	$< 0.20''$	—					

Table 2 Double point model for east subcomponent derived from 8.1-GHz data

Separation	Position angle	Flux ratio	Total flux density
$0.16 \pm 0.03''$	$121 \pm 10^\circ$	2.3 ± 0.3	0.66 ± 0.10

measurement errors the elongation of the radio structure therefore lies along the projected minor axis of the galaxy.

Discussion

First consider whether the compact component should be interpreted as (1) the relic of a past event in the nucleus which simultaneously produced the outer components of 3C236, or (2) the product of recent nuclear activity.

It is unlikely that the compact component has been contained for a period exceeding $\sim 2 \times 10^7$ yr, the light travel across the whole source. Possible confining mechanisms¹⁰ include thermal pressure, ram pressure and inertia. They must provide a confining pressure which exceeds P_{min} , the minimum (equipartition) internal pressure in the subcomponents due to relativistic particles and magnetic field. For subcomponent A, $P_{\text{min}} \sim 10^{-8}$ dyne cm^{-2} and for subcomponents B and C, $P_{\text{min}} \sim 10^{-8}$ dyne cm^{-2} .

In the case of thermal confinement the pressure balance condition immediately yields a lower limit for the product of the density ρ and temperature T of the confining gas. Thus with $P_{\text{min}} = 10^{-7}$ dyne cm^{-2} , $\rho T > 2 \times 10^{-5}$ g cm^{-3} degree. Now if $T \gtrsim 10^7$ K the thermal velocities within the confining gas would exceed the escape velocity of the galaxy. Such a hot

the relativistic plasma is expelled must have remained constant to better than 4° over more than 2×10^7 yr. It is presumably the rotational axis of the galaxy or of some dense region inside its nucleus. Aligned recurrent expulsions are difficult to reconcile with the 'gravitational slingshot' model of Saslaw *et al.*¹⁴ in which massive objects are flung out in the rotation plane of the elliptical galaxy. Furthermore, the alignment of the radio components along the minor axis of the galaxy provides an additional argument against this mechanism.

One class of models requiring quasi-continuous nuclear activity, which recently received considerable attention, involves the continuous flow of energy from the nucleus to hot spots in the outer components of double radio sources¹⁵⁻¹⁷. This energy is efficiently transported in a narrow beam at relativistic speeds. All the schemes that have been proposed to produce such relativistic beams (rotating massive object¹⁵, Laval nozzles¹⁷ and so on) operate over distances $\lesssim 100$ pc. It is therefore unlikely that the entire 2 kpc 'compact' component of 3C236 could be associated with the production of the beam. But the radio emission could well be due to interaction of the beam with non-relativistic gas in the vicinity of the nucleus of the galaxy. A fraction of the bulk energy in the beam would then be randomised in the galaxy rather than in the outer regions of the beam and the galaxy would seem to 'glow' at radio wavelengths. In this case the detailed radio structure would merely reflect that of the entrained non-relativistic gas illuminated by the relativistic beam. Such a picture would be consistent with the remarkable alignment, the agreement between the angles subtended by the westerly 'cone of ejection'

Table 3 The major subcomponents in the radio nucleus of 3C236

	Half intensity diameters		Position angle	Position*		Flux densities (Jy)				Spectral index
	Major	Minor		East	North	8.1 GHz	2.7 GHz	1.4 GHz	0.4 GHz	
A	$\sim 0.6''$	$\sim 0.1''$	$126 \pm 9^\circ$	$-0.43 \pm 0.03''$	$+0.26 \pm 0.03''$					
B	$< 0.05''$	$< 0.05''$		$0.12 \pm 0.02''$	$-0.08 \pm 0.02''$	0.44 ± 0.10	0.73 ± 0.08	1.15 ± 0.15	2.6 ± 0.15	0.67 ± 0.05
C	$< 0.05''$	$< 0.05''$	$121 \pm 10^\circ$	$0.25 \pm 0.02''$	$-0.16 \pm 0.02''$	0.46 ± 0.05				
Total	$0.8'' \pm 0.1''$	$0.1''$	$121 \pm 4^\circ$	0.0	0.0	1.39 ± 0.10	2.05 ± 0.20	4.4 ± 0.20		0.61 ± 0.05
Outer						0.20 ± 0.05				
double	2,340''	60''	122.5 ± 0.5			1.10 ± 0.10	2.12 ± 0.04	3.2 ± 0.2	7.0 ± 0.3	0.65 ± 0.05

*Position is given with respect to 2.7-GHz centroid (epoch 1950) $\alpha = 10 \text{ h } 03 \text{ min } 05.396 \pm 0.003 \text{ s}$, $\delta = 35^\circ 08' 47.84 \pm 0.03''$.

gas could not be maintained within the galaxy. An additional argument for even lower temperatures is provided by the presence of the [O II] $\lambda 3727$ line in the optical spectrum of 3C236 (refs 11 and 12). Taking $T < 10^7$ K gives $\rho > 2 \times 10^{-22}$ g cm^{-3} . Since the confining gas must be present over distances of at least 2 kpc ($\sim 0.7''$), a total mass of $> 10^{11} M_\odot$ would be required. Also, such large densities, with a magnetic field strength comparable to the equipartition value of $\sim 4 \times 10^{-4}$ gauss, would result in Faraday rotation of $\sim 10^8$ rad and complete depolarisation of the source at centimetre wavelengths. Since this component has been found to be polarised by $\sim 2\%$ at 6 cm (ref. 13 and Baker, unpublished) we discount the possibility of thermal confinement.

Ram pressure confinement would need even higher gas densities. Scaling a velocity $< c$ for the outer components to the compact central component results in an expansion velocity $v < 100 \text{ km s}^{-1}$. Since $\rho v^2 > 10^{-7}$ dyne cm^{-2} , $\rho \gtrsim 10^{-21}$ g cm^{-3} for confinement.

At first sight inertial confinement by a relatively cold gas inside the subcomponents seems more promising. Again taking $v < 100 \text{ km s}^{-1}$, this gas must have a total mass $> 2 \times 10^8 M_\odot$, corresponding to a density $\gtrsim 5 \times 10^{-26}$ g cm^{-3} . Since, for these densities also no polarisation should be measurable, inertia cannot provide the confining pressure. We have therefore shown that the compact central component of 3C236 is not confined and is unlikely to be a remnant of the event which gave birth to the large double radio source.

It is almost certainly the result of a relatively recent outburst in the nucleus of the galaxy. In this case, the axis along which

in the compact and extended structures and the observed asymmetry.

A more detailed picture of the compact component requires sensitive observations with even higher resolution. Measurements with VLBI techniques may reveal whether any of the subcomponents discussed here have even smaller scale features. Structure on a scale of $\lesssim 0.01''$ might relate to the origin of the radio event. In addition, an intensive optical investigation of 3C236 should be made. Photographs with a positional accuracy of $0.1''$ could be usefully compared with our radio data. Together with a spectroscopic study of the distribution of [O II] $\lambda 3727$ such observations should enable a clearer picture to be obtained of the dynamics and physical conditions within this remarkable galaxy.

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Ligand-induced redistribution of lymphocyte membrane ganglioside GM1

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Dynamic aspects of the binding of cholera toxin to lymphocyte membranes have been studied. We have shown that the receptor for this ligand—the GM1 ganglioside—can be laterally redistributed into aggregates and caps. Exogenous purified GM1 inserted into GM1-deficient human leukaemic cells can undergo a similar pattern of ligand-induced mobilisation. These observations may have important implications for both the general behaviour of cell surface glycolipids and the mode of action of cholera toxin on adenyl cyclase.

INTERACTION of divalent or polyvalent ligands (for example, antibodies, lectins) with cell surface structures on lymphocytes and many other cell types induces redistribution of the largely diffuse binding sites into clusters and into highly polarised single aggregates or 'caps'^{1,2}. The capping process is not fully understood but depends on active cellular metabolism and is probably regulated by microfilaments and microtubules³. These observations along with species antigen intermixing in mouse-man heterokaryons² provide visually dramatic evidence for a fluid mosaic model of cell membranes⁴. Lateral mobility of both proteins and lipids (which have similar diffusion constants) has also been demonstrated amply by electron spin resonance and electron magnetic resonance studies (reviewed in ref. 2).

A principal implication of membrane fluidity is clearly that translational movement induced by physiological ligands may facilitate molecular interactions that play a critical role in signal transduction across membranes.

Lipids may play a crucial role in cell surface receptor function by regulating molecular mobility and also by virtue of selective hydrophobic association with integral membrane proteins^{5,6}. The marked deficiency of several glycolipids, particularly the charged gangliosides, in virus and carcinogen-transformed animal cells⁷, in human leukaemia cells⁸, and in normal cells at particular phases of the cell cycle⁹, suggests that these lipids have an important role in growth control.

The ganglioside GM1 (monosialo-gangliotetraosyl-ceramide) is the natural receptor for cholera toxin¹⁰⁻¹² and it is now well established that cholera toxin (cholera toxin) exerts both its clinical effects (intestinal hypersecretion and diarrhoea) and its experimental effects (for example, induced steroidogenesis, inhibition of proliferation) through the activation of adenylate cyclase and accumulation of cyclic AMP¹³⁻¹⁵. Cuatrecasas and colleagues have proposed that lateral diffusion of cholera toxin-GM1 complexes within the plane of the membrane may lead to interaction with adenylate cyclase and that GM1 might play an important role not only in general growth regulation

but also in the coupling of hormone-induced responses¹⁶. Similarly, Field has suggested that cholera toxin may (through GM1) stabilise a catecholamine-sensitive conformation of adenylate cyclase in the turkey erythrocyte membrane¹⁷.

So far no demonstration of ligand-induced redistribution of a glycolipid has been reported. In view of the intriguing relationship between GM1 and adenylate cyclase it would be of considerable interest to determine conditions for redistribution of this glycolipid into aggregates and caps. A comparison of ganglioside and glycoprotein redistribution might reveal important implications for functional associations of gangliosides and proteins and information of relevance to signal transduction mechanisms in membranes. We demonstrate here, using cholera toxin and labelled antibodies as ligands, that GM1 on lymphocytes can indeed be redistributed into aggregates and caps, and moreover that GM1 inserted into GM1-deficient cells undergoes a similar pattern of ligand-induced redistribution.

Capping of lymphocyte membrane ganglioside

The experimental system for visualising the binding of cholera toxin to the lymphocyte surface is a three-layer immunofluorescence procedure (see legend to Fig. 2). Observations were performed using a Zeiss ultraviolet microscope with Plume, incident illumination and also the fluorescence activated cell sorter (FACS-1, Becton Dickinson, Mountain View, California)^{18,19}.

All lymphocytes, monocytes and granulocytes studied bound cholera toxin to their surface and uptake was rapidly saturated at 20 °C. Binding was inhibited by purified GM1 (Fig. 1). The biologically inactive cholera toxin bound almost as effectively as cholera toxin—as shown in other cell systems and expected from its competitive antagonistic capacity. At excess cholera toxin concentration there was no apparent difference between thymocytes, T and B lymphocytes; however, murine lymphocytes bound considerably more cholera toxin than human cells. We have shown previously that cholera toxin inhibits the proliferation *in vitro* of mouse T and B lymphocytes and L1210 lymphoma cells within a concentration range of 10⁻³–10⁻⁴ µg ml⁻¹ (ref. 8). The proliferation of human peripheral lymphocytes, however, was enhanced by these doses of cholera toxin, and only higher doses inhibited their mitogen-induced proliferative response.

Figure 2 illustrates the pattern of GM1 distribution observed on mouse and human cells incubated with different concentrations of cholera toxin. When all sequential steps were performed at 4 °C or at room temperature in the presence of 0.2 M sodium azide the distribution of fluorescence was either in aggregates of various sizes or, at higher concentrations (particularly on mouse cells), as intense rings. When all steps were performed at 37 °C on human lymphocytes very little surface fluorescence was detectable. We interpreted this observation as indicating that cholera toxin had been internalised rapidly or shed, and this view was supported by the following observations. When

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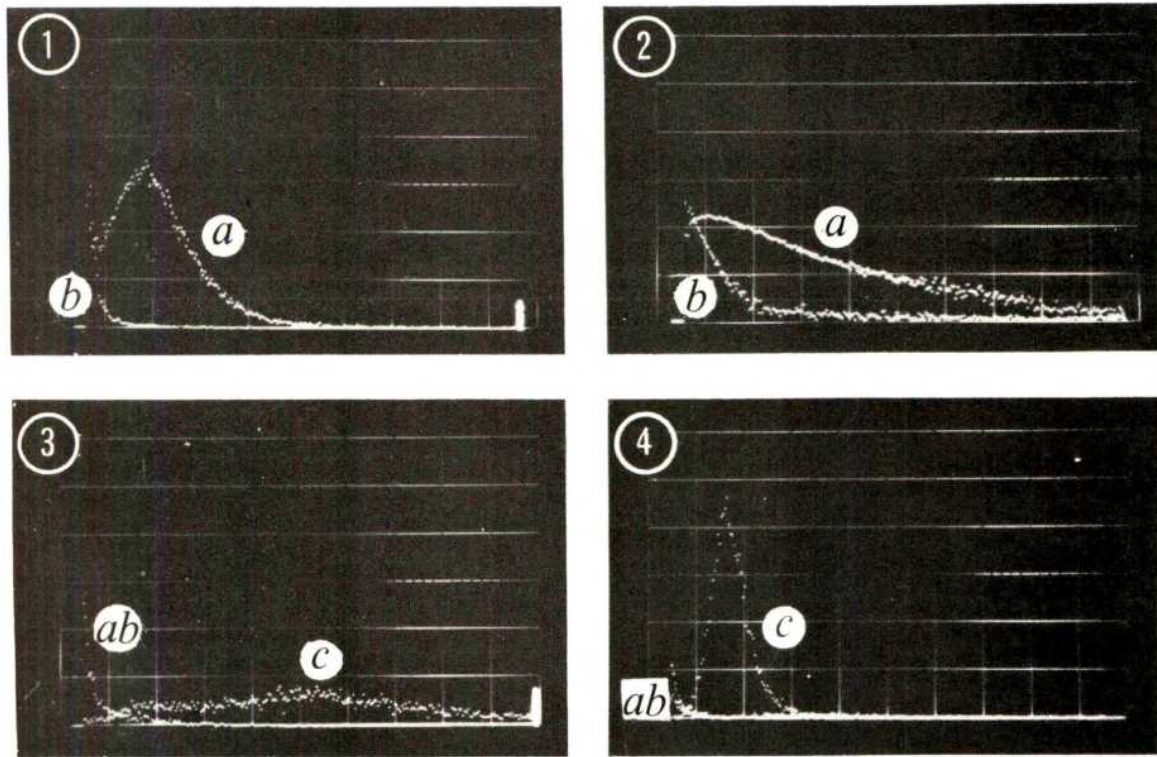


Fig. 1 Fluorescence activated cell sorter (FACS) analysis of cholera toxin binding to normal lymphocytes and leukaemic cells. The essentials of the immunofluorescence method are outlined in the legend for Fig. 2. Abscissa, relative fluorescence intensity; ordinate, relative number of cells, 10,000 cells counted in each sample. 1, Human thymus lymphocytes: *a*, cholera toxin; *b*, control (horse anti-cholera toxin and rabbit anti-horse- γ -fluorescein-isothiocyanate only). 2, Human tonsil lymphocytes: *a*, cholera toxin; *b*, cholera toxin preincubated with purified GM1 ganglioside. 3, Acute myeloblastic leukaemia cells: *a*, and *b* (superimposed), as in 1; *c*, cholera toxin binding after pretreatment of AML cells with neuraminidase (10 IU ml^{-1}). 4, Acute lymphoblastic leukaemia cells: *a*, and *b* (superimposed), as in 1; *c*, cholera toxin binding to cells that have incorporated exogenous GM1 (purified from brain—gifts from Drs R. Murrey and D. Critchley) into their surface membrane.

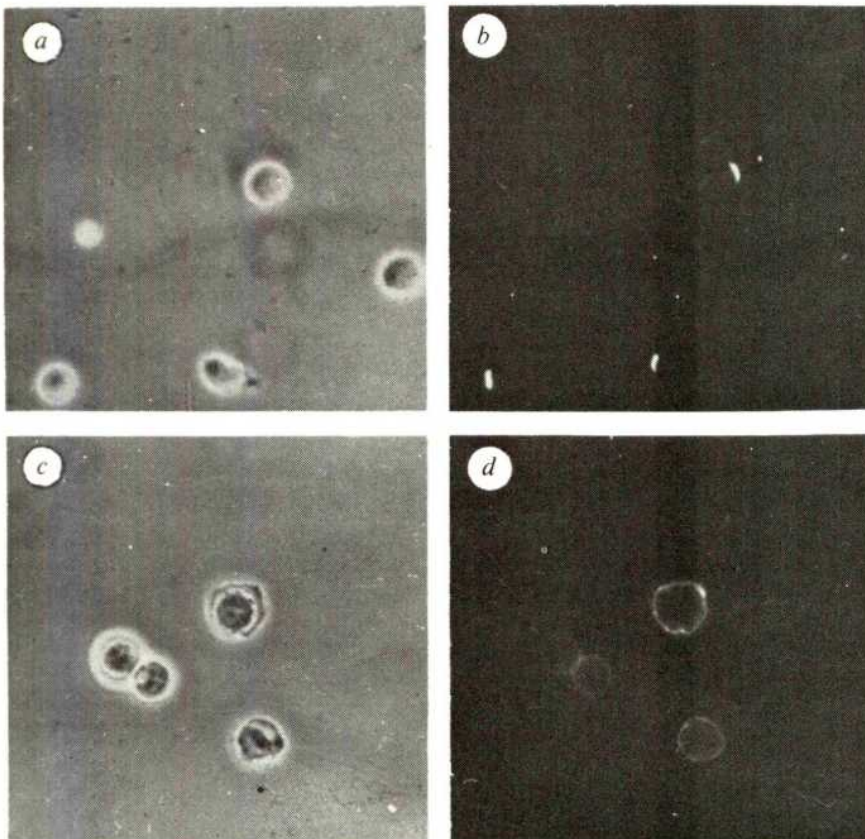


Fig. 2 Surface fluorescence pattern of human and murine lymphocytes labelled with cholera toxin. Cells in 0.2 ml Earl's saline ($2 \times 10^7 \text{ ml}^{-1}$) were incubated with cholera toxin ($100 \mu\text{g ml}^{-1}$) for 10 min at 4°C . After washing the cell suspensions twice, horse anti-cholera toxin serum was added in a final dilution of $1:10$, and the cells were incubated at 21°C for 30 min . Further washing was followed by a $1:10$ dilution of rabbit anti-horse- γ -globulin-fluorescein, isothiocyanate, and incubation at 37°C for 30 min . Washed cell suspensions were mounted on slides and examined using a Zeiss Ultraphot II microscope modified for epifluorescence microscopy. Photographs were taken with a Pentax camera. ($\times 524$). *a*, Human tonsil cells (phase contrast); *b*, human tonsil cells (fluorescence); *c*, murine spleen cells (phase contrast); *d*, murine spleen cells (fluorescence).

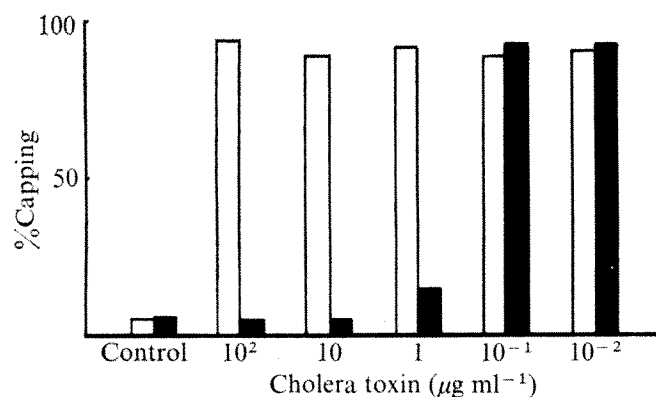


Fig. 3 Capping of cholera toxin on human and murine thymocytes. Lymphocytes in 0.2 ml Earl's saline (2×10^7 ml $^{-1}$) were incubated with various doses of cholera toxin for 10 min at 4 °C. Horse anti-cholera toxin was added to give a final concentration of 1:10, and cell suspensions were incubated at 21 °C for 30 min. The third incubation—with rabbit anti-horse- γ -fluorescein isothiocyanate was carried out at 37 °C for 30 min. Caps were defined as cell surface fluorescence restricted to one third or less of the cell surface. Open columns, human; black columns, mouse.

cholera toxin was added at 4 °C and either or both of the antibody layers at 37 °C, intense surface fluorescence was visible. The pattern of fluorescence as shown in Fig. 3 depended on the concentration of cholera toxin used and the species origin of lymphocytes. Human lymphocytes showed pronounced redistribution into discrete caps over a wide range of concentrations (compare Fig. 2) in contrast, mouse lymphocytes only showed caps with the lower concentrations used. Significantly, the biologically inactive cholera toxin gave essentially the same results as cholera toxin. By incubating mouse spleen lymphocytes with low concentrations (10^{-4} – 10^{-2} µg ml $^{-1}$) of cholera toxin at 37 °C followed by the two antibody layers at 4 °C in the presence of azide it was possible to demonstrate capping on approximately 50% of reactive cells. Although the intensity of cap fluorescence was less than when the second and/or third steps were performed at higher temperatures (compare Fig. 2), this result demonstrated that cholera toxin itself can induce redistribution into caps. We suspect that the lack of observable capping of GM1 on mouse lymphocytes treated with high concentrations of cholera toxin is related to a higher level of GM1 in these cells compared with human lymphocytes. Although we have not yet quantified cholera toxin binding sites on these cells, this interpretation is supported by the observation that capping of GM1 on human cells (at high cholera toxin concentrations) is inhibited by creation of more GM1 binding sites for cholera toxin. This was achieved both by neuraminidase pretreatment—which converts other gangliosides (that is GD1a, GT1; di- and tri-sialo-gangliosides) into GM1 by cleaving off sialic acid residues²⁰—and by direct insertion of GM1 into cells (Table 1).

Table 1 Inhibition of capping of GM1 on human tonsil cells

Pretreatment	% Capping
None	89.5
Trypsin (0.05%)	85.0
Neuraminidase (10 IU)	2.2
GM1 insertion (20 µg)	3.0
Colchicin (10^{-4} M)	86.5
Cytochalasin B (10 µg)	48.2
Colchicin (10^{-4} M)+ cytochalasin B (10 µg)	27.6

Human tonsil cells (2×10^7 ml $^{-1}$) were incubated with trypsin, neuraminidase and purified GM1 at 37 °C for 30 min followed by cholera toxin at 4 °C, horse anticholera toxin at 21 °C and rabbit anti-horse- γ -globulin-fluorescein-isothiocyanate at 37 °C. Colchicine and cytochalasin B were added to the appropriate cell suspensions 5 min before each step. Caps were defined as cell surface fluorescence restricted to less than one third of the cell surface.

Holmgren and colleagues recently reported on the distribution of mouse thymus lymphocyte GM1 using a triple layer system essentially the same as that reported here²¹. They observed both ring staining and aggregation, but in contrast to our results, no cap formation. The reported concentration of cholera toxin used in these experiments, however, was 1 µg ml $^{-1}$. As can be seen from Fig. 3, this dose is considerably above the maximal concentration for cap induction, in mouse lymphocytes.

Capping of immunoglobulin molecules on lymphocytes is inhibited by a combination of colchicine and cytochalasin^{22,23}. Although these drugs may have diverse effects on cells, these studies suggest that microfilaments and microtubules have an important role in molecular mobility in the membrane³. Significantly, these drugs have precisely the same effect on GM1 redistribution (Table 1); in combination they are more potent than either alone. Although GM1 is the biologically relevant receptor for cholera toxin and free GM1 can completely inhibit binding to cells (see above and refs 11 and 12), cholera toxin does bind at least weakly to other gangliosides and also to some glycoproteins. It was therefore theoretically possible that the capping we observed was not due to GM1 redistribution but to movement of other structures. The following experiment provides, however, an unequivocal demonstration of ganglioside redistribution into caps.

Gangliosides inserted into membranes

Many transformed animal cell lines are ganglioside deficient⁷, and we have reported that virtually all human acute leukaemia cells have a considerable deficiency in binding sites for cholera toxin⁸. These cells efficiently incorporate exogenous GM1 into

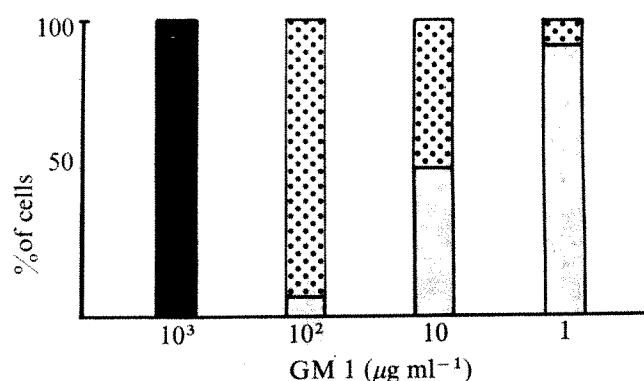


Fig. 4 Insertion and capping of purified GM1 in human acute lymphoid leukaemia cells. Leukaemia cells in 0.2 ml Earl's saline (2×10^7 ml $^{-1}$) were incubated with various doses of purified GM1 for 30 min at 37 °C. After washing the cells twice in Earl's saline cholera toxin (100 µg ml $^{-1}$) was added, followed by two antisera, as described in the legend for Fig. 2. Fluorescence staining patterns: Solid columns=ring (continuous surface staining—see Fig. 2); dotted columns=patchy (fluorescence aggregates of various sizes); stippled columns=caps (surface fluorescence restricted to less than one-third of the cell surface).

their membranes and bind cholera toxin^{8,24} (Fig. 1); alternatively, if they are treated with neuraminidase, cholera toxin binding occurs, presumably by the conversion of other gangliosides to GM1 (Fig. 1). We have performed similar experiments with the rodent cell lines BHK and NIL; however, because these cells are by chemical criteria deficient in all gangliosides²⁵ they can be rendered cholera toxin-reactive by GM1 insertion but not by neuraminidase treatment. After either GM1 insertion or neuraminidase treatment acute leukaemia cells show pronounced capping provided that not too much GM1 is inserted (Fig. 4); a result predicted by our earlier observations (compare Fig. 3). Although it is theoretically possible that GM1 insertion reveals endogenous non-GM1 binding sites for cholera toxin,

we regard this as extremely unlikely and therefore suppose that all the redistribution we observe is due solely to lateral mobilisation of the inserted GM1. These experiments provide compelling evidence for the lateral redistribution of glycolipids.

Possible mechanisms of glycolipid redistribution

The mechanics and molecular requirements for glycolipid redistribution are of course unknown. It is however possible to conceive of two basically different means by which a glycolipid such as GM1 might become capped. It may do so by association with integral proteins or a particular protein. Alternatively, glycolipids on motile lymphocytes might be in continual orientated motion, cross linkage by ligands then resulting in 'trapping' at the rear 'pole' of the cell. At present we have no unequivocal evidence which clearly supports either of these alternatives; distinguishing between them will perhaps have important implications for the dynamics and functional roles of lipids in cell membranes. The capping process is insensitive to trypsin (Table 1) and so we assume that if GM1 did have an obligatory association with an integral protein then the latter structure is trypsin insensitive—at least as far as its facilitative interaction with GM1 is concerned.

Since exogenous GM1 molecules inserted into membranes can be redistributed instantly we must also assume that any specific conditions necessary for mobilisation would have to be established rapidly by newly incorporated molecules. It is particularly relevant in this respect that at least some of the GM1 molecules and other glycosphingolipids incorporated into the membrane of glycolipid-deficient transformed cells can alter growth characteristics²⁶ and render cells sensitive to the biological effect of cholera toxin²⁴. The aggregation of GM1 molecules into clusters and caps which is induced by 'physiological' concentrations of cholera toxin may be very relevant to the regulation of adenylate cyclase activity as suggested by Cuatrecasas and colleagues. But since the biologically inactive cholera toxinoid has virtually the same binding capacity as cholera toxin and can also induce redistribution of GM1 (see

above) it would seem that aggregation of GM1 molecules is not in itself sufficient to alter adenylate cyclase activity.

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Genetic perturbations that reveal tertiary conformation of tRNA precursor molecules

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The ubiquity of tRNA-like conformations in tRNA precursors of prokaryotes provides a common structural basis for precursor recognition by the maturation enzymes. This design eliminates the need for multiple enzymes to achieve the maturation of different precursors. Moreover, the requirement of this specific conformation for maturation guards against the production of potentially deleterious mutant tRNAs.

THE salient features of tRNA biosynthesis in prokaryotic organisms are fairly well understood. The process begins by transcription of a tRNA gene to yield a large precursor RNA, which is then converted into tRNA by a series of enzymatic steps that include the modification of specific nucleotides and cleavage of the precursor RNA. The absence of inaccurately processed precursor RNAs in normal cells demonstrates the accuracy of this multi-step process. Such specificity must be determined in part by precursor RNA conformation, so it is at this level of complexity that we must investigate the mole-

cular mechanism that guarantees accurate tRNA synthesis. Precursor RNA conformation and its relation to tRNA biosynthesis are the subjects of this report.

The original observation on which our experiments were based is that mutationally altered forms of phage T4 glutamine tRNA contain reduced levels of modified nucleotides¹. Since modification depends on conformation, these mutants might be used for indirect study of precursor RNA conformation. Modified nucleotides are formed at the level of precursor RNA by enzymatic modification of one of the standard nucleotides. Comparison of many tRNA sequences indicates that the formation of modified nucleotides rarely if ever involves enzymatic recognition of the primary nucleotide sequence alone². These considerations and other kinds of evidence³ indicate that recognition relies on the secondary and possibly tertiary conformations of the RNA molecules. The lack of modified nucleotides in mutant glutamine tRNAs therefore implies the existence of distorted conformations of the corresponding precursor RNAs, which contain glutamine and leucine tRNA sequences^{4,5}. These considerations raise an interesting question. Are deficiencies in nucleotide modifications

confined to the glutamine tRNA region of the precursor RNA or are they also present in leucine tRNA? This obviously will depend on the specific conformation of the precursor RNA at the time its nucleotide residues are modified, as illustrated by the following example. If there is little or no interaction between the residues of the two tRNA sequences, as would be the case with two cloverleaf secondary structures, then only glutamine tRNA will be deficient in nucleotide modifications. By contrast, if there are extensive interactions between the residues of the two tRNA sequences, then the modified residues of both tRNAs will be deficient in the mutants. We have tested these possibilities by isolating mutant precursor RNAs and determining their content of modified nucleotides. As viewed by this kind of analysis the conformation of the precursor RNA involves little or no interaction between the residues of the two tRNA sequences.

Mutant-specific patterns

Precursor RNAs labelled with ^{32}P were purified to more than 80% radiochemical homogeneity by two successive steps of polyacrylamide gel electrophoresis. They were then analysed for modified nucleotides. Table 1 gives the percentages of modification at twelve positions in the glutamine-leucine precursor RNA that is synthesised after infection of *E. coli* by the psu_2^+ ochre-suppressing strain of phage T4. psu_2^+ is the structural gene of an ochre-suppressing glutamine tRNA¹. A value of 100% in

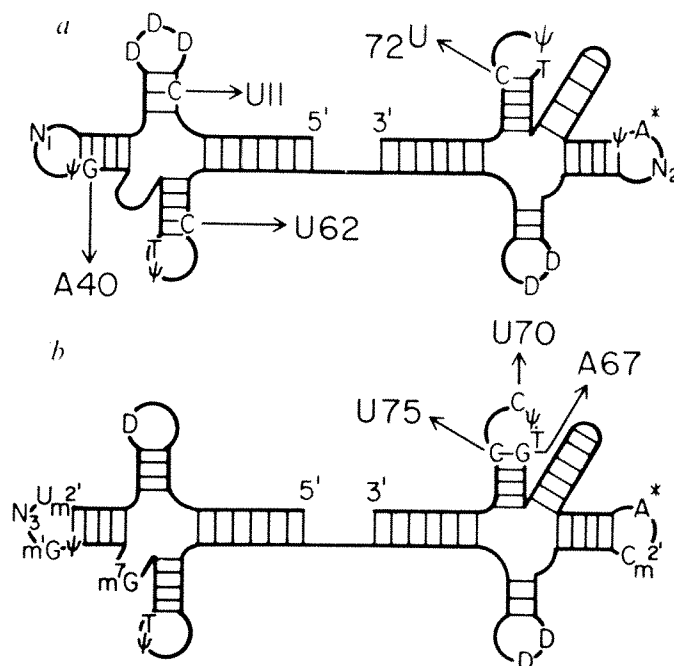


Fig. 1 Positions of modified and mutant nucleotides in glutamine-leucine (a) and proline-serine (b) precursor RNAs. Other residues are omitted. The phosphate-sugar backbone of the tRNA sequences is designated by the thick continuous line that is arranged in a double cloverleaf conformation. Hydrogen bonds involved in maintaining the cloverleaf conformations are designated by the short thin lines that are parallel. The 5' to 3' polarity of the RNA chains is indicated. Residue N_1 at the top left is in the anticodon of the psu_2^+ glutamine tRNA sequence¹, and N_2 on the right is in the anticodon of leucine tRNA¹⁷. At the bottom left N_3 is in the anticodon of the proline tRNA sequence^{6,7}, whereas $Cm^{2'}$ and A^* bracket the anticodon of the psu_1^+ serine tRNA^{6,9}. Arrows show nucleotide substitutions in the individual mutant of psu_2^+ (top) or psu_1^+ (bottom). All mutants have been described previously^{1,4,9} except for $72U$, a newly-isolated mutant of psu_2^+ (our unpublished results).

Table 1 Percentage of nucleotides that are modified in glutamine-leucine precursor RNAs

Glutamine tRNA nucleotide (position)	RNase fragment analysed	psu_2^+		$psu_2^+ - U11$	
		1 (%)	2 (%)	1 (%)	2 (%)
D (16)	P13	96	84	11	7
D (17)	T11	86	66	3	3
D (20)	T6	113	89	65	55
N_1 (34)	T17	43	63	< 2	< 2
Ψ (39)	T17	87	108	103	97
T (54)	P17	68	92	13	24
Leucine tRNA					
nucleotide (position)					
D (16)	p4	112	97	95	79
D (20)	u11	64	61	53	57
N_2 (35)	u15C	32	28	44	38
A^* (38)	u15C	25	31	24	33
Ψ (40)	p12	92	85	83	93
T (65)	p15	123	87	77	91

Standard nucleotide abbreviations³ are used except that N_1 and N_2 refer to the modified residues of unknown identities that are located in the anticodons of glutamine¹ and leucine¹⁷ tRNAs, respectively. In the first column position refers to the number of residues that a nucleotide is removed from the 5' phosphate of the mature tRNA sequence. The ^{32}P -labelled precursor RNAs were isolated after infection of the ribonuclease P-deficient strain A49¹³ and were purified by polyacrylamide gel electrophoresis^{7,10}. Wild-type B/5 strain was used as the host for psu_2^+ mutant $72U$ (Fig. 1), since this precursor RNA does not accumulate in A49 cells (our unpublished results). Modification percentages were determined by analysing the RNase A or T₁ fragment, derived from a two-dimensional fingerprint, that is indicated (fragment designations are defined elsewhere^{1,12}). The analyses involved digesting the appropriate fragment to mononucleotides with RNase T₂, fractionating the products by two-dimensional, thin-layer chromatography and then comparing the amount of ^{32}P radioactivity in modified and unmodified residues⁷. Modified residues at a level of 100% contained about 400 c.p.m.; the lower limit of detection was about 2%. Different precursor RNA preparations were used for experiments 1 and 2. In precursor RNA, glutamine and leucine tRNAs both contain the sequence GT Ψ CG; values for the Ψ s of these two sequences were not determined because the two Ψ s are in identical RNase A and RNase T₁ fragments and therefore cannot be distinguished. No evidence of modification (that is, < 2%) was observed for glutamine tRNA residue m^2A (position 37) or for leucine tRNA residues D (position 17) and $Gm^{2'}$ (position 18).

Table 1 means that all the precursor RNA molecules contain the modified residue in question. Values that fall between 2%, the lower limit of detection, and 100% reflect heterogeneity in the precursor RNA preparation with respect to a particular modified residue. Table 1 shows that the values obtained with two preparations of precursor RNA are fairly reproducible for most of the residues. We do not know if the observed fluctuations reflect genuine differences in the precursor RNA preparations or inaccuracies in the measurements. For our purpose these fluctuations are small and do not affect the validity of the major conclusions presented below.

Table 1 shows a similar set of duplicate measurements for strain $psu_2^+ - U11$. The loss of psu_2^+ ochre-suppressing activity in this strain results from a C to U mutation at the 11th nucleotide from the 5' end of glutamine tRNA¹. We see that many of the modified residues are reduced in amount or are entirely absent in the $psu_2^+ - U11$ precursor RNA. Note the asymmetry in the reductions and that they are confined to the part of the precursor RNA sequence containing glutamine tRNA.

Further evidence for localisation of the effect of a mutation is provided by analysis of three additional mutants of psu_2^+ . Mutants $psu_2^+ - A40$ and $psu_2^+ - U62$ involve nucleotide changes in glutamine tRNA¹, whereas mutant $psu_2^+ - 72U$ has a C to U change at position 72 in leucine tRNA (our unpublished results). The change in notation with strain $psu_2^+ - 72U$ indicates that the mutant nucleotide is not in the sequence of the glutamine suppressor tRNA. Precursor cleavage yielding glutamine and leucine tRNAs is drastically reduced in mutant $psu_2^+ - 72U$, which explains why this strain lacks the suppressing activity of glutamine tRNA. The top panel of Fig. 1 gives the relative positions of mutant and modified nucleotides in the glutamine-leucine precursor RNA. Figure 2 is a graphic representation of

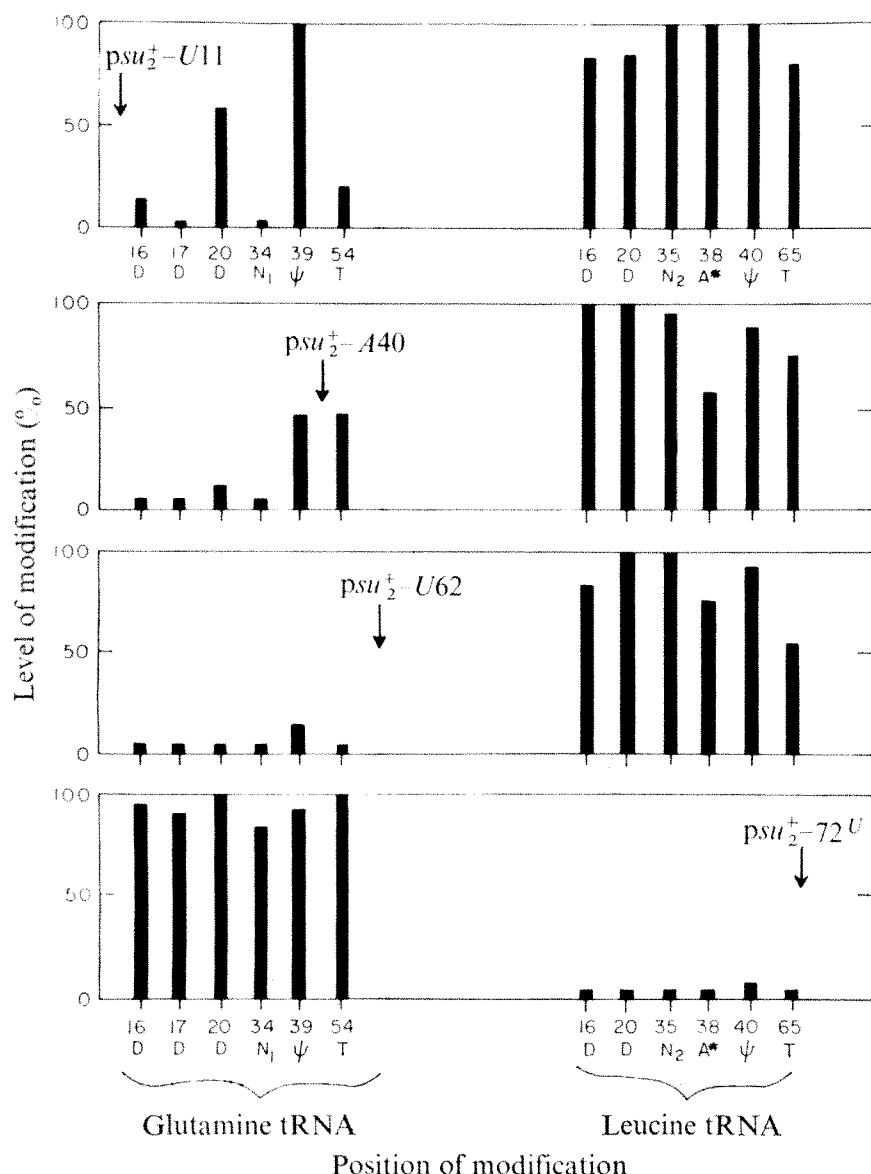


Fig. 2 Patterns of modified nucleotides in mutant glutamine-leucine precursor RNAs. The top panel displays the data in Table 1. The horizontal axis arranges the modified nucleotides with their respective residue numbers such that left to right corresponds to the 5' to 3' polarity of the RNA chain. The vertical axis gives the levels of modifications as percentages of the psu_2^+ values. The arithmetic means of the duplicate determinations were used. The vertical arrow in the top panel shows where the *U11* mutation is located relative to the modified residues. The remaining panels at the bottom show analogous results obtained from single determinations with three other mutants of psu_2^+ .

the modification levels in the four psu_2^+ mutant precursors compared with the level of the corresponding residue in the psu_2^+ precursor. The figure shows that deficiencies are confined to the tRNA sequence containing the mutant nucleotide. This is a clear demonstration of the independence of the two tRNA sequences at the time nucleotide modifications are introduced into the precursor RNA.

We obtained analogous results with the proline-serine precursor RNA that is synthesised after T4 infection^{6,7}. psu_1^+ is the structural gene of an amber-suppressing serine tRNA^{8,9}. Three suppressor-negative mutants of psu_1^+ involving nucleotide substitutions in serine tRNA (Fig. 1, bottom panel) were examined. Predictably, the mutant precursors were only deficient in nucleotide modifications in serine tRNA (Fig. 3).

Double cloverleaf conformation

The results in Figs 2 and 3 show that the major reductions in nucleotide modifications of precursor RNAs are confined to the tRNA sequence bearing the mutant nucleotide. There is no evidence that a mutation can have a significant effect on the formation of a modified nucleotide in the neighbouring portion of the precursor RNA containing the non-mutant tRNA sequence. These results demonstrate an independent substrate structure of the two tRNA sequences, and thus provide strong indirect evidence that the conformations of the precursor RNAs are predominated by interactions between the residues of the

individual tRNA sequences. Although the data do not specify the specific residue interactions, we suspect that they are quite similar to those found in the mature tRNAs. Such a conformation seems likely because both precursors contain only a few nucleotides in addition to those of the mature tRNAs⁵⁻⁷. We do not know the tertiary conformations of the tRNAs under consideration. The secondary structures are, however, sufficiently defined to allow for the proposal that the glutamine-leucine precursor RNA and the proline-serine precursor RNA will consist of pairs of tRNA-like cloverleaf entities as shown in Fig. 1. Although our results strictly refer to the conformations at the time of nucleotide modification, it is likely that a similar folding will characterise the molecules subsequently. Incidentally, the importance of the tertiary interactions within each cloverleaf to the substrate structure of the tRNA is documented by the absence of numerous modified nucleotides in the mutant tRNA sequences (Figs 2 and 3).

Several additional features of Figs 2 and 3 require comment. The first is the fractional modification of some residues (for example, glutamine tRNA residues D16, D20, Ψ 39, and T54 in mutant psu_2^+-U11). These patterns are mutant specific and presumably reflect the presence of residual precursor RNA conformations that are sufficient for modification of only a fraction of the precursor RNA chains. Such an explanation may be less likely when the same residue is fractionally modified in two or more mutants (for example, glutamine tRNA residue

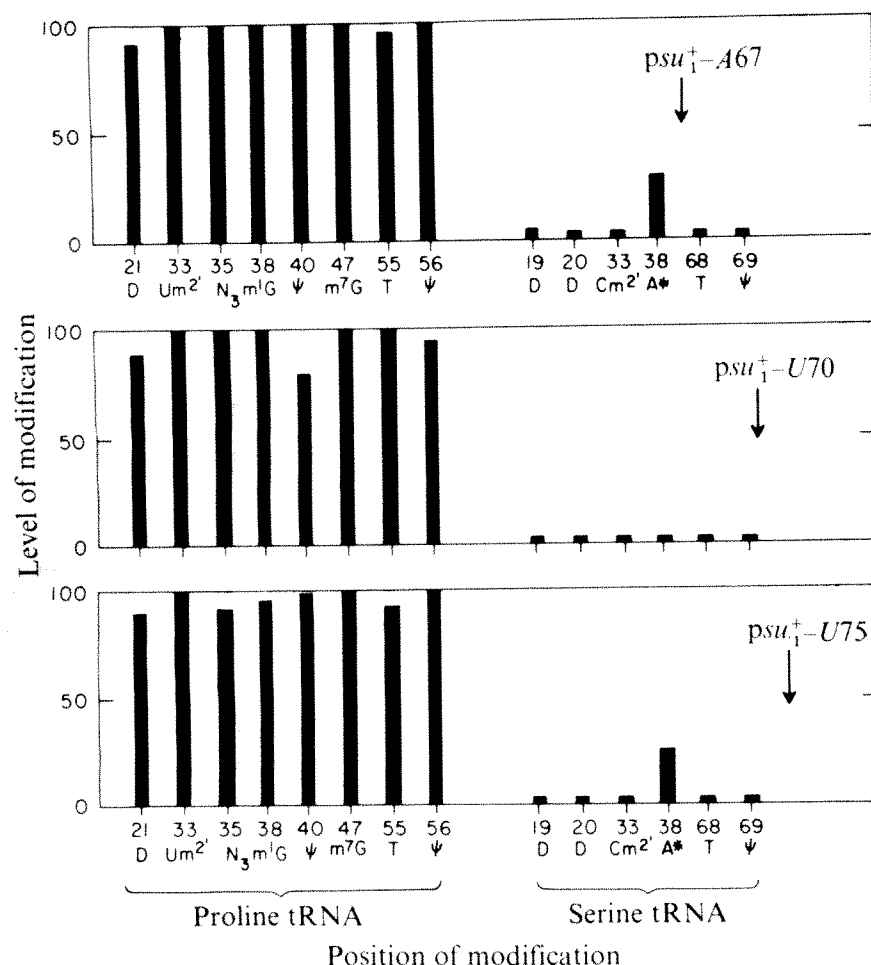


Fig. 3 Patterns of modified nucleotides in mutant proline-serine precursor RNAs. The levels of modified nucleotides are expressed as percentages of the values obtained with the psu_1^+ precursor RNA. All results were obtained from single determinations. The psu_1^+ values were similar to those reported previously⁷.

Ψ39 and serine tRNA residue A*38). In these cases either the recognition sites for the modifications are somewhat smaller and less specific in detail than with other residues, or there is an excess of these modification enzymes. Finally, there are indications in Figs 2 and 3 of slight reductions in nucleotide modifications in the non-mutant tRNA sequence (for example, leucine tRNA residues D16, A*38, and T65 in mutant psu_2^+ -U62). We do not know if these result from trivial inaccuracies in the measurements or reflect tertiary interactions between the two cloverleaf entities that influence the extent of modification. The latter possibility is potentially quite exciting, since it might offer an approach to the study of tertiary conformations of the precursor RNAs. Further experiments are in progress.

Implications for tRNA biosynthesis

Our proposal that precursor conformation closely resembles that of the mature tRNAs provides a molecular basis for understanding some of the precursor RNA biosynthetic details elaborated previously. Cleavage by ribonuclease P in the central region of the proline-serine precursor RNA is dependent on a specific nucleotide sequence at the distant 3' end of the RNA chain¹⁰. The secondary interactions of the cloverleaf bring these two distant regions of the RNA chain together, thereby enabling a single ribonuclease P molecule to interact with them as substrate for cleavage.

More generally, we suspect that a tRNA-like conformation will characterise many prokaryotic precursor RNAs. Consequently precursors of entirely different nucleotide sequences will nevertheless have similar conformations. This kind of conformational uniformity provides a common basis by which the numerous maturation enzymes can specifically recognise and handle many different precursor RNAs. The potential genetic economy that this provides is significant, since only a small amount of DNA is needed to specify an array of enzymes for maturation of many precursor RNAs. Our proposal is sup-

ported by several types of experimental results. Chang and Smith¹¹ have shown by *in vitro* chemical reactivity of the surface nucleotides that the precursor to *Escherichia coli* tyrosine tRNA has the same conformation as mature tyrosine tRNA. Unlike the phage T4 precursors described above, the tyrosine precursor contains a single tRNA species, but its maturation nevertheless involves the participation of ribonuclease P (ref. 12). Moreover, conditionally lethal mutants of *E. coli* defective in ribonuclease P fail to synthesise all tRNA species in the non-permissive conditions, and instead accumulate precursor RNAs containing one or more tRNA sequences¹³. Thus ribonuclease P is involved in the processing of precursor RNAs of diverse sizes and sequences, and it is presumably able to accomplish this by recognising the tRNA conformations in the precursor RNAs. In addition, accumulation of many different precursor RNAs in the absence of other participating maturation enzymes has also been demonstrated^{10,11,15}. Finally, the requirement of a native conformation in the precursor RNA for a productive interaction with the maturation enzymes is demonstrated by what happens when mutations like those shown in Fig. 1 destroy the conformation: they block the conversion of precursor RNA into tRNA^{1,8,9,16}. Such a safeguard for eliminating potentially deleterious tRNAs before they are functional may in part explain why precursor RNAs were implemented for the synthesis of tRNAs.

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letters to nature

Candidate for LMC X-5

A NEW X-ray source in the Large Magellanic Cloud, LMC X-5, has been reported¹. I wish to draw attention to the close proximity of the radio sources MC32 and MC33 to the best X-ray position. According to the positions of MC32/33 given by McGee *et al.*² they lie just outside the X-ray 90% confidence level error box for LMC X-5. In Fig. 1 I show the 6-cm radio contours of MC32/33 from ref. 2 with the X-ray error box superimposed on a plate of the region.

MC32/33 is one of six double radio sources found in the LMC by McGee and Newton³ to have one thermal and one non-thermal component. The non-thermal component (MC32) has a radio spectral index of -0.64 between 6 and 73 cm, not very different from the average value of -0.48 found by Milne⁴ for galactic supernova remnants. The association of another of these double radio sources, MC76/77, with the X-ray source LMC X-1 has previously been suggested⁵. This latter suggestion has been subsequently strengthened by X-ray observations of improved positional accuracy⁶.

Optically MC32/33 is identified with the H II region N44 of Henize⁷. Isophotes of the northern half of N44 (MC32) by

Dickel⁸ show it to have a ring-like structure which may strengthen the supernova interpretation of the object. LMC X-5 is probably a highly variable X-ray source as evidenced by the failure to detect it with Uhuru. This would imply that the X rays are emitted by a compact object (such as a neutron star) which might be expected in association with a supernova remnant.

The region around N44 is rich in early-type stars. Many X-ray sources have been identified with binary stellar systems, one component of which is a compact object and the other an early type star. One star is of particular interest—No. 73 in Sanduleak's⁹ list (indicated in Fig. 1 as Sk73) of LMC early-type stars between -68° and -69° declination. Spectroscopic observations by Feast *et al.*¹⁰ (their No. R99) indicate an emission spectrum with no visible absorption spectrum. Contamination by nearby nebulosity is an unlikely source of the emission features as the nebulosity is extremely weak in the immediate area of Sk73. I note that the He II $\gamma 4686$ line is present, a line which is frequently observed in emission in the X-ray binary stars. Photoelectric observations^{10,11} do not indicate any large amount of variability but the star may have an ultraviolet excess for its $(B-V)$ colour¹¹. Photographic photometry carried out on ADH plates (loaned by Armagh Observatory, N. Ireland) do not indicate variability in B magnitude of more than 0.15 mag.

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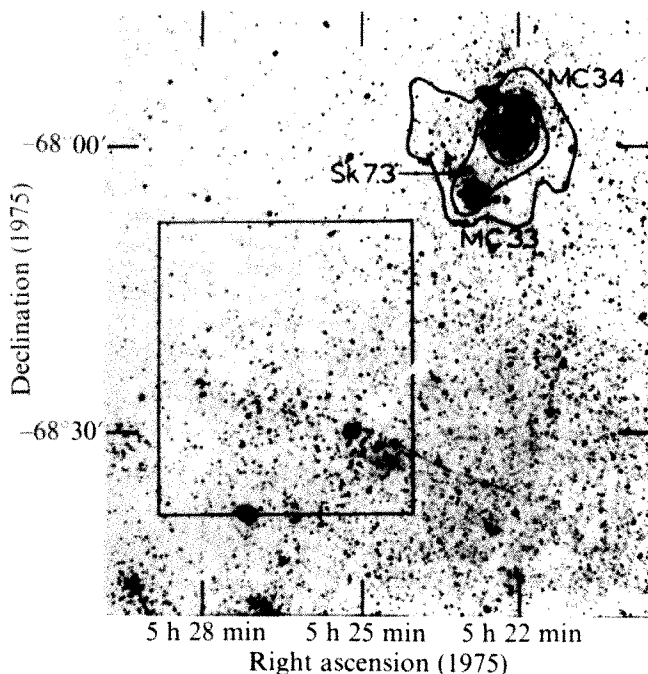


Fig. 1 The X-ray error box for LMC X-5 with the 6-cm radio contours (at intervals of 0.1 K antenna temperature) superimposed on a B plate of the region.

Internally heated convection in the atmosphere of Venus and in the laboratory

IN this letter I draw attention to the possibility that the cellular convection observed by Mariner 10 in the subsolar region of the atmosphere of Venus may be related to laboratory studies of convection with internal heat generation.

Significant regions of the Venusian convection show cellular arrays^{1,2} indicative of some form of "Bénard convection", in the general sense of convection deriving from instability of the vertical temperature gradient. The fact that the convecting region is roughly centred on the subsolar point suggests that

this unstable stratification is generated directly by solar radiation. The Venera 8 observations³ of the attenuation of sunlight with decreasing altitude show that most of the radiation absorbed by the planet is in fact absorbed in the cloud layers. On the other hand, they also show that an earlier view that it might all be absorbed very close to the cloud tops was incorrect, as is, in any case, implied by the occurrence of convection. A detailed distribution of the absorption with respect to altitude cannot be inferred from the Venera 8 observations, because of the unknown relative importance of absorption and scattering in different layers⁴. But it is highly probable that the convection is being driven by solar heating within the convecting fluid. Although cellular convection is commonly associated with the convecting layer being heated from below, it is now known to occur also in internally heated layers. Moreover, in an important respect (that is, the "elongation" of the convection cells), the Venusian convection has a closer resemblance to laboratory convection of the latter type than of the former.

The "elongation" of the convection cells means that their horizontal scale is large compared with the depth of the convecting region. The cells in the Venusian atmosphere have a horizontal size¹ (presumably to be interpreted as about twice the distance between fastest rising and fastest falling fluid) of typically 200 km; some are as large as 500 km. The top of the convecting region is thought to be at an altitude^{1,5} of 63 km. It is possible that the convection extends down to the surface of the planet, but more likely it is shallower. The Venera 8 observations³ indicate that there is no significant heat absorption below an altitude of 35 km; further analysis of these observations⁴ places the bottom of the absorbing region at any altitude between 35 km and 48.5 km. It is also possible⁵ that the temperature gradient is subadiabatic below about 45 km, although evidence on this point is contradictory³. One should further take into account the fact that the scale height of the atmosphere (about 15 km) is not large compared with the other lengths involved. The effect of this is not well understood. It will presumably tend to reduce the vertical size of the convection cells—since, in order of magnitude, one expects this size to be the smaller of the scale height and the depth of the unstably stratified region—but the modifications to the flow may be relatively minor⁶. In summary, therefore, it is apparent that, whatever method one uses to infer the vertical scale of the convection, this scale is certainly much smaller than the horizontal scale of the larger cells and probably significantly smaller than that of a typical cell. This contrasts with previous suppositions about the likely structure of convection in the Venusian atmosphere⁷.

Correspondingly, in laboratory studies of cellular convection, it was supposed that the horizontal and vertical dimensions are normally roughly equal until experiments were performed with internally heated layers⁸. Convection cells in the classical situation of a fluid layer heated at a lower boundary and cooled at an upper boundary do grow horizontally with an increasing Rayleigh number⁹, but not so much as to affect seriously the above supposition. Experiments^{8,10} with an internally heated layer, thermally insulated at the bottom and cooled at the top, give a much more dramatic growth of horizontal cell dimensions. Cells with the centres of the rising and falling regions separated by a distance of five times the layer depth have been observed, although a factor of three may be considered more typical. (A more detailed study of this situation has been made (T. Hooper and D. J. T., unpublished); some of the statements in this letter draw on those results.) Similarly elongated cells have been observed in experiments¹¹ with boundary conditions such that the convecting region lies above a stably stratified region, a case in which both horizontal and vertical cell dimensions are free to adjust.

(It should be mentioned that the interpretation of some of the experiments has given rise to some controversy¹², primarily due to the failure of theoretical work to predict the observed elongation. My reasons for believing that the observations nevertheless provide the best starting point for comparison

with natural situations will be developed elsewhere. In summary, it may be said that most of the theoretical calculations were done in a way that could not show the effect or else contained oversimplifying assumptions. A qualitatively similar, although quantitatively smaller, discrepancy between theory and experiment exists in the case of a fluid layer heated from below and cooled from above; in that case, possible causes of the disagreement on the experimental side have been much more carefully eliminated.)

It thus seems likely that, despite the small scale height and other probable complexities of the former, the basic dynamics of the Venusian convection and of the laboratory convection are similar. Quantitative comparison would imply that the larger cells in the Venusian atmosphere extend down to close to the surface, whereas the smaller ones may be vertically limited in one of the ways mentioned above. But it may well be that greater elongations than have been observed in the laboratory can occur—the dynamics of all sufficiently elongated cells being broadly similar—and thus that the convection is entirely confined to an upper atmospheric layer. Elongated cellular convection is observed in the laboratory only for a range of the Rayleigh number; we are thus supposing that small scale turbulence ("eddy viscosity" and "eddy conductivity") provides dissipative effects of the appropriate magnitude, but such a supposition is necessitated by the observation of any type of cellular convection.

Comparison of other aspects of the Venusian and laboratory convection shows general consistency. Some of the Venusian cells are polygonal, others have a more streaky appearance. In the laboratory, both polygonal and roll-like cells have been observed over different Rayleigh number ranges. The Venusian cells have highly structured interiors. In the laboratory, cells with an elongation (as defined above) of greater than about two develop an internal structure; a prominent feature is the intermittent ejection of cold blobs of fluid from the region close to the upper boundary, similar to those found in recent computational studies¹³. But these features do not sufficiently distinguish this type of convection from other types for them to be taken as supporting the main thesis of this letter.

If developing understanding of the Venusian atmosphere confirms the idea that the convection is, in its basic dynamics, similar to the laboratory convection, this may have significant implications for other situations. In particular, it may encourage application to the interior of the Earth, for which the relevance of the laboratory experiments is at present a matter of discussion¹³⁻¹⁶.

The fact that mesoscale convection in the Earth's atmosphere exhibits elongated cells¹⁶ provides a further point of comparison. The dynamical processes involved are not understood, although it is usually supposed that the elongation results from anisotropy of small scale turbulence. It is worth noting, however, that a model of mesoscale convection involving volume heat sources due to latent heat release has been developed¹⁷. The model is very different from the laboratory situation considered above—in that the heating is non-uniform and cooling due to evaporation is also involved—but it does lead to the speculation that the laboratory experiments may have closer relevance to the Earth's atmosphere also than was previously supposed⁸.

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Duration of equatorial spread F

It is well established¹⁻³ that the spread-F phenomenon, which is observed as diffuse echoes on the ionograms is a common feature during night time between the dip latitudes 20°N and 20°S. Based on the study of ionograms and published monthly f_oF_2 bulletins, morphological features of spread F have been reported for several equatorial stations^{1,4-10}. Equatorial spread F as seen on the ionograms generally starts around the time of reversal of the F-region vertical motion in the evening and

continues to grow in intensity during the downward motion of the F region¹¹. Usually spread F persists only for few hours and on some occasions continues throughout the night. Sometimes, after the disappearance in the pre-midnight period, spread F reappears in the post-midnight period.

We have examined the published ionospheric data of Trivandrum (dip latitude 0.6°S, Geographic Longitude 76°52'E) for the year 1970 to study the factors influencing the duration of spread F and the results are presented in this communication. The duration of spread F is determined by using the ionograms taken at 15-min intervals. The parameter $h'F$ is taken to represent the height variations of the F region. In the year 1970 there were about 30 nights in which spread F started in the evening hours (≈ 1900 IST) and continued until the early morning. The $h'F$ variations with time have been examined for all these nights. Figure 1 shows a plot of the hourly variation of $h'F$ for ten typical long duration spread-F nights. Plotted in the same graph are the $h'F$ variations for some of the nights in which spread F was present only for 2-3 h, to compare with

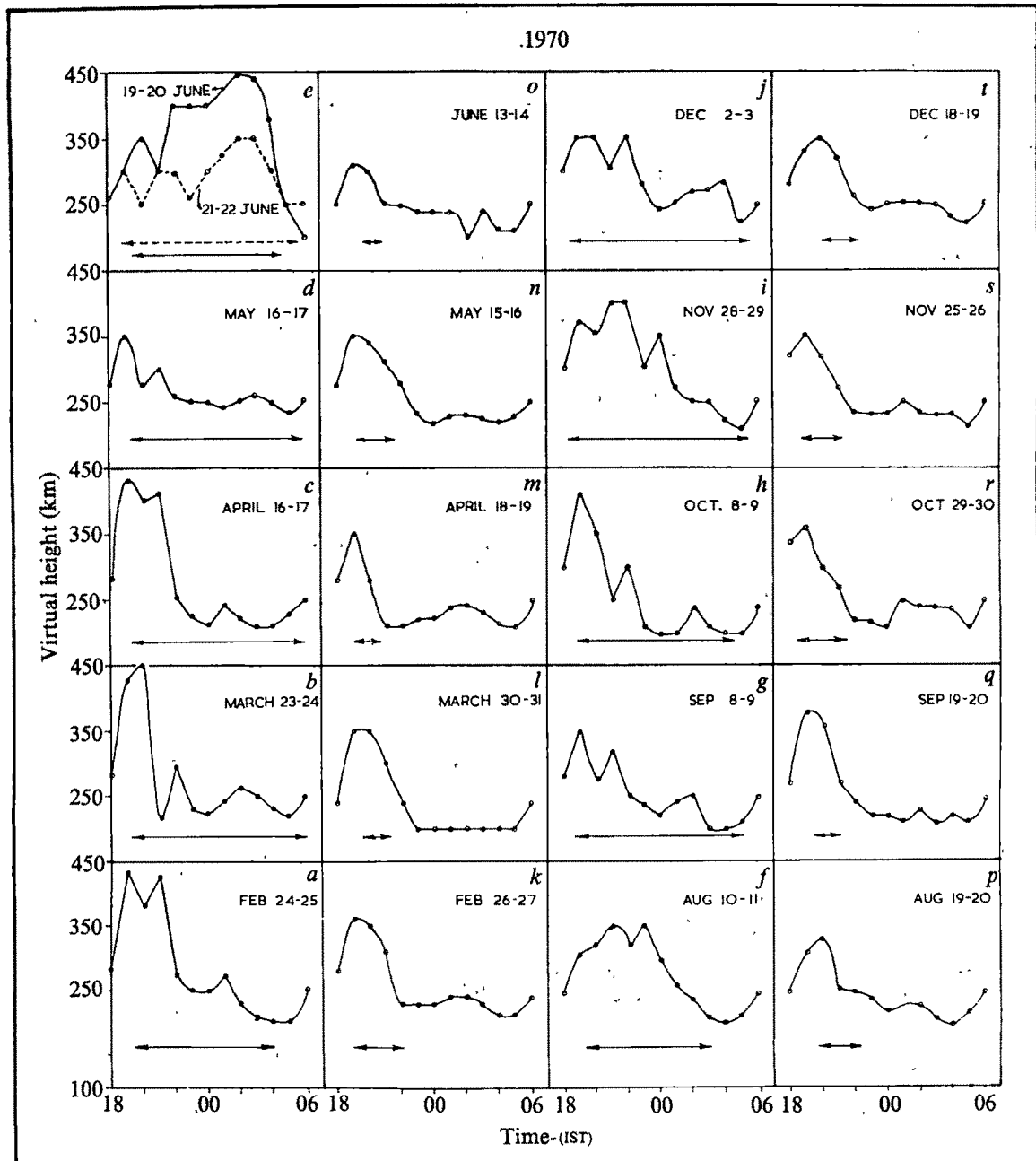


Fig. 1 $h'F$ variations during short and long duration spread-F nights. a-j, Long duration spread F; k-t, short duration spread F (2-3 h).

the $h'F$ variations during long duration spread-F nights. The duration of spread F is indicated by a line with arrows in these graphs. All the nights considered correspond to undisturbed magnetic conditions as indicated by the K_p index. Further, we have considered in this study only those nights with continuous duration of spread F. The main features of Fig. 1 are:

(1) $h'F$ starts rising from 1800 IST on all the nights shown and the onset of spread F is generally around the time of maximum $h'F$.

(2) The most striking feature is that on nights of long duration spread F, $h'F$ shows significant fluctuations. In many of these cases $h'F$ shows two peaks separated by about 1–2 h and another peak generally in the post-midnight period. On the other hand, on nights with short duration spread F, $h'F$ shows only a single maximum around 1900 IST and does not show any significant fluctuations. This is a consistent feature observed on all the nights in the year 1970 when spread F is present.

(3) On all the nights shown in Fig. 1, $h'F$ reaches approximately the same maximum height irrespective of the duration of spread F except in four cases.

(4) It is interesting that on August 10–11, 1970 (Fig. 1f) spread F continued for relatively shorter duration compared with other long duration nights and on this night, the $h'F$ fluctuations are not as prominent as they are on other nights of long duration spread F.

From an examination of the $h'F$ data on spread F nights for the whole year, it is found that even when the $h'F$ peak is above 400 km, the duration of spread F is only a few hours. Once the spread-F irregularities occur and there is no sustaining mechanism of the irregularities, these will decay in a time corresponding to the chemical loss time constant. To examine this on a quantitative basis, the chemical loss time constants are estimated corresponding to the average $h'F$ during 1900–2000, for the night of short duration spread F. For this estimation, the chemical loss coefficients given by Mitra¹² and

fluctuating east–west electric field forms one of the prerequisites of spread F generating and or sustaining mechanisms. This is consistent with the suggestion made by Hanson *et al.*¹⁴ that turbulent electric fields in the F region could be a necessary condition for equatorial spread F.

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Short term relationships between solar flares, geomagnetic storms, and tropospheric vorticity patterns

It has been found that the 500–mbar vorticity area index (VAI), which is a rough measure of the strength of the cyclonic activity over the Northern Hemisphere, responds to solar flares in at least two ways. First, there is an increase in the VAI in the first two days after the flare. Then, after the geomagnetic storm which often follows a major solar flare, there is a sharp decrease in the VAI. The results are consistent with earlier studies of the development of pressure troughs in the North Pacific area.

Many investigators have reported short term relationships between variable solar activity and weather, but the relationships have often been unconnected and even contradictory. The results reported here seem to form a logical extension of other results recently reported^{1–4}.

Roberts and Olson⁴ verified an earlier finding regarding wintertime pressure troughs at the 300 mbar level, and their apparent response to a solar-geomagnetic signal. If a day of sudden increase in geomagnetic activity is called a geomagnetic key day and the day that a trough moves into or is formed in the Gulf of Alaska is called the 0-day of the trough, they verified that the following relationship held: troughs whose 0-days fell 2–4 d after a key day were selectively intensified, on the average, during the next few days. The day of maximum intensity was about 1 d after day 0, or about 4 d after the key day.

In this and all previous work, it was very crucial that 2–4 d must elapse between the key day and day 0 for the effect to be observed. If day 0 was less than 2 d after the key day, the intensification of the trough was, on the average, missing or even negative. The geomagnetic key day was defined as any day on which the planetary geomagnetic index, A_p , equalled 15 or more, and on which the one day increase in A_p from the previous day exceeded the monthly average A_p . Thus it was defined by the sharp front of an abrupt rise in geomagnetic activity.

To measure the size and intensity of the troughs, a VAI was devised. This is defined as the area over which the absolute

Table 1 Observed and estimated spread-F data

Date (1970)	Average height (km)	Chemical loss time constant (h) (calculated)	Duration of spread F (h) (observed)
February 26–27	355	2.8	2.0
March 30–31	350	2.5	1.75
April 18–19	315	1.9	1.1
May 15–16	345	2.2	2.2
June 13–14	305	0.75	1.0
August 19–20	290	0.5	2.0
September 19–20	370	4.4	2.0
October 29–30	330	1.3	2.5
November 25–26	335	1.7	2.3
December 18–19	335	1.7	2.3

CIRA 1965 model have been used. These are shown in Table 1 along with the observed duration of spread F. There is a fairly good agreement between the observed duration of spread F and the calculated chemical loss time constant. It should be noted that the duration of spread F depends on the initial strength of the irregularities as well as on the chemical loss time constant. Considering this, the agreement between the two is quite satisfactory.

The night-time $h'F$ fluctuations represent the true height (h) fluctuations corresponding to the undersurface of the F region¹³. The variation of $h'F$ (or equivalently of the true height h) with time represents the sum of the layer velocity and the apparent upward velocity caused by the chemical loss. The latter is not expected to show the type of fluctuations in $h'F$ observed in the present investigation (Fig. 1). As such, the observed fluctuations in $h'F$ should correspond to the true vertical motion of the F region, which at the Equator is attributed mainly to the electrodynamic drift caused by an east–west electric field. From this we contend that the $h'F$ fluctuations (observed on long duration spread-F nights) indicate similar fluctuations in the east–west electric field. We suggest that the

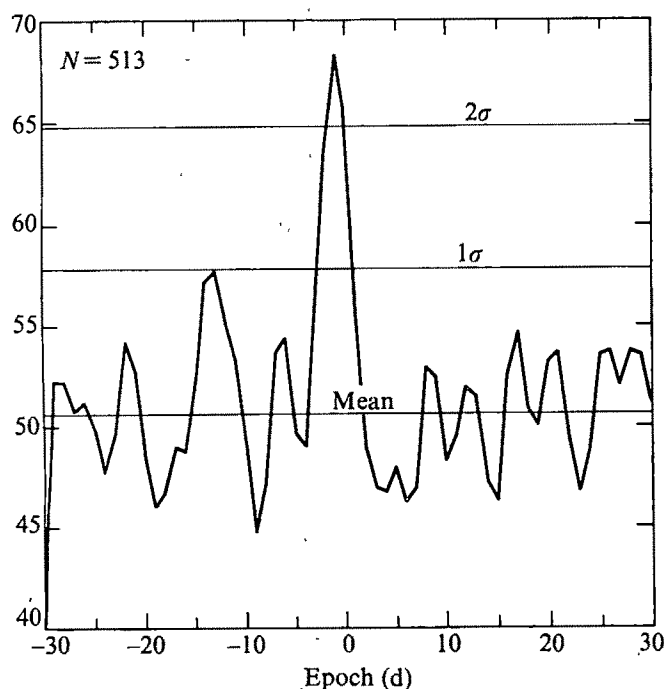


Fig. 1 Number of geomagnetic key days before and after zero days established by the three minimum value days per month of the 500 mbar VAI September–April, 1950–71.

vorticity exceeds certain threshold values. We have applied the same VAI definition to both 300 mbar and 500 mbar. We base the work in this paper on our 500-mbar results rather than those at 300 mbar, because the data at 500 mbar are more complete and homogeneous.

Wilcox *et al.*¹⁻³ have introduced a new solar signal into the study, namely the interplanetary magnetic sector boundaries that sweep past the Earth several times per month, apparently due to the Sun's rotation and the fact that they are imbedded in the solar wind and locked to magnetic field configurations on the solar surface. They also modified the VAI somewhat. Instead of matching VAI patterns with individual troughs, they integrated the VAI over the entire Northern Hemisphere north of 20°N. This makes the integrated VAI a completely objective parameter, whereas the VAI applied to individual troughs involves the degree of subjectivity necessary to identify specific troughs with vorticity maxima.

The Wilcox results, both at 500 and 300 mbar and with several independent data samples, showed a very consistent pattern. Approximately 1 d after the Earth intersects a solar sector boundary, the integrated VAI displays a 10% decrease. By 3–4 d after the crossing, the VAI has recovered to its original level or even beyond it.

Since there is a well known tendency for geomagnetic disturbances to occur near the time of sector boundary crossings, it was tempting to do a study similar to the Wilcox studies, but using geomagnetic key days as the solar signal. This report gives the results of such an experiment, plus a similar one in which major solar flare occurrence was used as the solar signal.

One of the difficulties in working with VAI data is that, although the VAI can be computed objectively by computer, the map sets needed as a basis for the calculations are somewhat non-homogeneous and therefore produce noisy data. Thus we decided in the first experiment not to use the VAI itself, but to select the three days per month when VAI was a minimum as the meteorological parameter. We then performed a superposed epoch analysis (SPE) of the number of geomagnetic key days preceding and following the three days of minimum vorticity.

The frequency of key days reaches a sharp maximum 1 d before the minimum vorticity (Fig. 1). The 1σ line on Fig. 1

was computed by the assumption of a Poisson distribution; it is the square root of the average number of key day occurrences so computed. Therefore, according to Langley⁶ the significance levels are conservative. By this estimate, the peak at day -1 is significant at almost the 1% level. Langley points out that "The Poisson answer is nearly always a shade weaker than the true binomial probability, so there is very little risk of getting a 'significant' answer when you shouldn't." It may be of interest that Langley also gives examples (ref. 6, pp. 249–250) of testing of similar distributions which give results much like ours. In addition the high value at day 0 is significant at better than the 5% level. It must be noted that not all 513 cases in the sample are independent—the days of minimum vorticity sometimes occur in clusters or even at times may be consecutive. But it is not certain that this detracts from the significance of the result. If the effect of a geomagnetic event is such that it causes several consecutive days to have low VAI values, this may support the reality of the effect.

The second part of the study involves a correlation between the occurrence and magnitude of major solar flares and the integrated VAI. Dodson and Hedeman⁵ have shown that, from a list of all well observed major flares, only the upper 20% or so in magnitude have a clear-cut causative relationship to magnetic storms. Thus in our study we used only the flares from the Dodson–Hedeman list having a combined index of 10 or more. This gave a list of 94 flares in the winter half year and 115 in the summer half, for the period 1955–1969 covered by the Dodson–Hedeman list. As a preliminary experiment we used the days of minimum vorticity, as in Fig. 1, to establish day 0, and performed an SPE analysis of flare index from day -30 to day 30.

The results (not shown here) showed only one peak, a highly significant increase in flare index at day -3. But this experiment did not add much to our knowledge, because of the well known relationship between major flares and geomagnetic disturbance. To show the relationship between solar flares and VAI in a more explicit way, we prepared Fig. 2. First, in order to remove the seasonal trend in the VAI, we subtracted each daily value from the 29-yr mean for that day. An alternative way of taking out the seasonal trend would be to subtract each daily value from a fairly extensive running mean value, for instance 27 d. We tried that technique, and again got results quite similar to those shown in Fig. 2. Even with this trend removal procedure, there is still considerable noise in the VAI data, as mentioned earlier. We performed an SPE analysis with the 0 dates determined by the occurrence of a solar flare of index 10 or more (roughly the upper 20% in flare magnitude). The VAI data appear in the form of 24-h changes, so that the data shown at the 0 point is the 24-h change in the integrated VAI from day 0 to day 1.

To test the statistical significance of the results we have plotted them in the form of Student's *t* values. The results are somewhat unexpected. In the winter half year, Fig. 2 shows a significant decrease in integrated VAI, from three to four days after the flare. This is just what we would expect, in view of Fig. 1 and the fact that these are the flares that typically produce geomagnetic storms about two to three days after the flare. But an even more significant increase in VAI occurs on the first and second day after the flare, a surprising result, since we had not seen evidence for this in other data. Although the noise level in Fig. 2 is high, we feel that both the sharp increase and the subsequent decrease in VAI are real. The increase seems significant on both days 1 and 2 following the flare. As in Fig. 1, we know of no way to incorporate the position of the peaks into a significance test. That is, if the most significant peaks and troughs occurred at day -18 or day 22, for instance, we would be hard pressed to ascribe a causative relationship to them. But since they occur near day 0, we feel that the suggestion of a physical connection must be taken seriously. The fact that a similar relationship (not shown) was found in independent data from summer months, even though it was weaker, must also be considered as evidence for the reality of the finding.

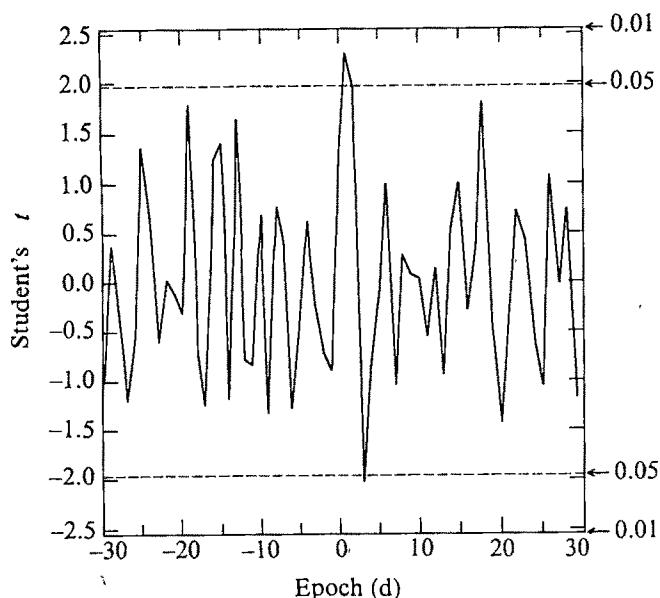


Fig. 2 Student's t values for 24-h change in Vorticity Area Index (expressed as departure from mean) before and after 94 large flares, 1955–69 (winter season, October–March). Arrows on right indicate labelled confidence limits.

From this work it is possible to suggest an average sequence of events, as follows (applicable primarily, but by no means exclusively, to the upper troposphere and to the winter half year). Starting with the day of a large solar flare as day 0:

- (1) By day 1 or 2, the Northern Hemisphere VAI increases sharply, by 5–10% above its background value;
- (2) by day 2 or 3, a geomagnetic storm has started, which may persist for several days;
- (3) by day 3 or 4, the VAI has decreased to a value 5–10% below its level before the flare;
- (4) by day 5 or 6, the VAI has recovered back to its original level.

The results of Fig. 2 can also be used to suggest a reason for the trough intensification in a large homogeneous area like the North Pacific following a geomagnetic key day. Immediately following the geomagnetic key day the integrated VAI is depressed. It is generally found that troughs in the North Pacific reflect the general hemispheric situation—they tend to be smaller when the integrated VAI is small. By 3 d after the key day, the hemispheric VAI is increasing, and the Pacific troughs apparently share in this tendency.

These results, of course, are true only in the average, and should not be taken to be case studies. Moreover several additional refinements suggest themselves. For instance, we should examine the position of the flare on the disk of the Sun as a factor in determining the VAI response. It is undoubtedly important, and the type of response obtained may give a clue as to what parameter in solar activity is most important, the ultraviolet or X-ray flux, particles, and so on. It will also be vital to examine the geographic areas of greatest response. Answers to questions like these will aid greatly in the all important problem of discovering physical mechanisms to explain the observed results. Once we have reasonably acceptable physical models, we should be enabled to perform much more critical tests of the reality of solar-weather interactions.

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Crystal structures, the Earth and Dirac's Large Numbers Hypothesis

DIRAC's Large Numbers Hypothesis assumes that the large, dimensionless numbers obtained from the ratios between fundamental physical constants are related to the age, t , of the Universe^{1–4}. The theory demands two important consequences: that matter be continuously created with time and that the gravitational constant, G , vary with time. To reconcile those demands with Einstein's theory of gravitation two metrics are required. One, the Einstein metric, involves the equations of motion and classical mechanics; the other, the atomic metric, refers to quantum mechanics and laboratory measurements of distances and time as determined by atoms. According to Dirac, the continuous creation of mass requires either of two models—one, called additive creation, assumes that matter is created uniformly throughout space (mostly intergalactic space). The other model is called multiplicative creation. It calls for the creation of matter where matter already exists in proportion to the amount and kind already existing.

A corollary of the multiplicative creation model is that Einstein's cylindrical closed Universe suit cosmological considerations. Adopting the Einstein metric, and using the Einstein field equations, Dirac argues that if the radius of the Universe and G were constant and the galaxies were not receding then all atomic distances, expressed in Einstein units, would vary proportionally to t^{-1} . In terms of Einstein units, the number of atomic particles varies as t^2 whereas the mass of atomic particles varies as t^{-2} . In that way the mass of a classical body can remain constant. The fundamental atomic constants of physics all vary in a compensatory fashion, so, presumably, as 'new' atoms are added to 'old' atoms all atoms are changing so that at any point in time 'old' and 'new' atoms are indistinguishable.

Suggested tests of the theory³ remain unconfirmed. Measurements of astronomical distances with atomic apparatus could provide a test as could highly accurate, secular measurements of G . The Earth can also provide tests for the theory. Adopting the atomic metric, mass and atomic distances remain constant, but as G varies the Earth would expand and move away from the Sun^{1,5,6}. Continental drift and plate tectonics are, in part, consistent with this test of the atomic metric.

Crystals in rocks of the Earth represent a record of possible change from at least 3×10^9 years ago up to the present so their atomic structures should agree with the predictions of the theory. When dealing with atomic units no problem emerges as mass and atomic distance remain constant so no differences between old and new crystals can be expected since any variation in G would be negligible compared with the atomic forces involved. But when dealing with Einstein units major changes are demanded at the atomic level. Dirac² has stated that "There might be some difficulty in understanding how the matter in very old rocks can have multiplied without disrupting their crystal structure". If atomic distances vary as t^{-1} then the d -spacings in crystal lattices in geologically older minerals should

be observably different from those in the same minerals forming today. The geological evidence, however, is strongly against such a prediction: the lattice dimensions of a 3×10^9 yr-old quartz crystal are the same as the lattice dimensions of quartz grown in the laboratory. Indeed, the molecular weight and the X-ray density of the unit cells are the same. Therefore, unless Avogadro's hypothesis is violated, the number of atoms in the unit cell is also the same. The relevant equation from X-ray crystallography is:

$$V = Mn/\rho N_0 \quad (1)$$

where, respectively, V is the lattice volume, n is the number of atoms, M is the molecular weight, ρ is the X-ray density of the crystal unit cell, and N_0 is Avogadro's number. The equation must be valid in both atomic units and Einstein units. But no difference in any of these parameters is observable between an early Precambrian crystal and a modern crystal of the same composition. Therefore, in Einstein units, this condition violates the predictions of the multiplicative creation model.

Gittus⁸ has offered an apparent solution to this difficulty and has suggested that the 'new' atoms could occupy interstitial positions at vacancies and dislocations. Calculations show that for a crystal 3×10^9 yr old, if $n \propto t^2$ more than 30% of the atoms would have to occupy interstitial positions. Although this is clearly too many for any crystal to accept without lattice disruption it would, in any case, have to produce a marked difference between the X-ray and pycnometrically determined densities.

The geological evidence does not confirm the predictions of the multiplicative creation model and unless Avogadro's number is to be held constant with time, similar difficulties arise for the additive creation model. In its present form, therefore, the Large Numbers Hypothesis seems to be unacceptable.

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Origin of alkaline rocks

THE hypothesis of limestone assimilation^{1,2}, developed to explain differentiation of the Vesuvius magma, was long unchallenged for that area—and extended³—even when it had to be abandoned for other alkaline provinces. Trace element data made it difficult to reconcile the limestone assimilation hypothesis with many observations⁴⁻⁷, but even in 1966 when Hurley *et al.*⁵ challenged Rittmann's hypothesis¹ (on the basis of Sr isotope data) they had insufficient evidence to rule it out. But Pb isotope data support⁶ the genetic model of Hurley *et al.*⁵, and further evidence against Rittmann's hypothesis is presented here.

The main difficulty Hurley *et al.* encountered results from the overlap of the $^{87}\text{Sr}/^{86}\text{Sr}$ values found in limestone and in the alkaline rocks from Vesuvius and the other volcanic areas they investigated. The Sr isotopic composition of limestone is determined by two components: The Sr in the carbonate will have the isotopic composition of seawater at the time of deposition, assuming no later Sr exchange. Clay minerals in the limestone will generally have retained their inherited isotopic composition. Peterman *et al.*⁹ found a range in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio for seawater of 0.707–0.709 with the least radiogenic Sr

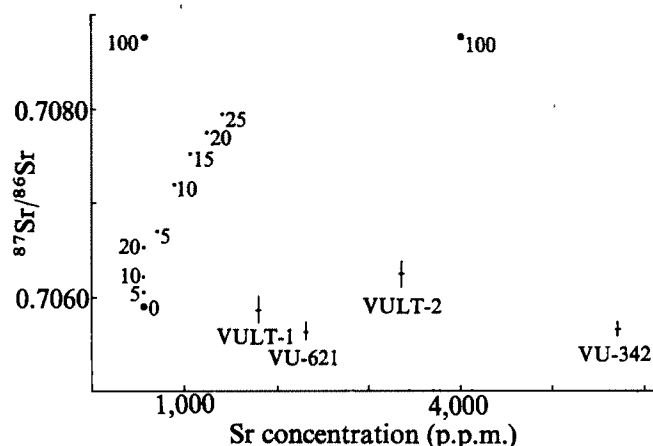


Fig. 1 The change in the Sr isotopic composition which would be produced by the assimilation of limestone with 610 p.p.m. Sr (average limestone¹⁴) or evaporites with 4,000 p.p.m. Sr (possible, though rather high value¹⁶) is plotted as percentage of assimilated sediment. The magma is assumed to have 550 p.p.m. Sr and an isotopic composition similar to the rocks from Vulture. The Sr isotopic composition of the sediments is assumed to be equal to Miocene seawater⁹. The data from Vulture are taken from ref. 8.

in the Jurassic and Cretaceous and a rapid increase in the ratio during the Tertiary. These will be minimum values. Impure limestone tends to have higher values due to inherited radiogenic Sr in the clay minerals. For example, average values of 0.7129 and 0.7141 for Jurassic and Cretaceous limestones were obtained¹⁰ as compared with 0.707 for pure limestone of the same ages. Impure limestone will have less radiogenic Sr than pure limestone only in the rare cases when a significant amount of the total Sr originates from volcanic material with non-radiogenic Sr derived from the mantle. Thus, an attempt to refute the limestone assimilation hypothesis will be facilitated, if the uncontaminated magma has a $^{87}\text{Sr}/^{86}\text{Sr}$ ratio below the minimum value for seawater. New Sr isotope determinations on the Italian alkaline rocks⁸ have shown that this seems to be the case for the Cainozoic¹¹ volcanic complex of Mt Vulture in Southern Italy. The assimilation of limestone¹² or Miocene evaporites¹³ has been suggested as an explanation for the occurrence of haüyne and leucite in rocks from Mt Vulture.

Assuming Sr concentrations for the uncontaminated magma and the sediment, the effect on the isotopic composition resulting from these hypotheses is demonstrated in Fig. 1. None of the predicted correlations can be observed. The range in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio is very small and the mugearite (VULT-2), which according to the hypothesis should have suffered little or no contamination, has in fact a ratio which is slightly higher than the rest of the samples. Furthermore, the haüynofyr (VU-342), which according to the hypothesis should have the highest portion of assimilated sediments, has the lowest isotope ratio, which is in contradiction to the hypothesis. Thus, a characteristic feature of these rocks—the very high Sr concentrations—cannot have been caused by the assimilation of a sediment.

As more Sr isotope data on the Italian alkaline rocks become known, a pattern emerges, which enables one to test Rittmann's hypothesis also at Vesuvius. The Sr isotope ratio is rather constant throughout the eruption sequence for each volcanic centre (with a total range generally less than 0.0012) and each centre has a distinct range. This indicates individual and quite uniform sources. The Somma-Vesuvius volcano seems to be the one exception. Here relatively large variations in $^{87}\text{Sr}/^{86}\text{Sr}$ which ranges from 0.7068 to 0.7100, are found. Even within the same lava flow almost the same spread can be observed: two samples from the 1944 lava flow yield 0.7072 (ref. 8) and 0.7095 (ref. 10). These exceptional large variations and the inhomogeneities within a single lava flow point to a superficial contamination with Sr. This might well be caused by the abundant

limestone inclusions which are subjected to various degrees of metamorphic alteration¹. But if Rittmann's hypothesis were correct, that increasing assimilation with time of dolomite and limestone would have produced the successive desilification of the Vesuvian lavas, the Sr isotopic composition would be expected to correlate with the eruption sequence and the silica content. Neither can be observed.

This line of argument only breaks down if the exceptionally large variations are not caused by superficial contamination, or the contamination has a different origin, for which no evidence has yet been put forward. Then any Sr contribution from the limestone inclusions would be masked and the Sr tracer method could not be used to detect any possible limestone assimilation at Vesuvius.

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Laboratory-induced self reversal of thermoremanent magnetisation in pillow basalts

CONSIDERABLE attention has been paid to the changes in the magnetic properties of pillow lavas with distance from a ridge¹. It has been found^{2,3} that the originally stoichiometric titanomagnetites are altered at sea-floor temperatures to cation-deficient titanomagnetites (titanomaghaemites). The degree of cation deficiency—the fraction of initial Fe^{2+} oxidised to Fe^{3+} —is described by the parameter 'z' (ref. 4). As the titanomaghaemite evolves, all the magnetic properties (for example, Curie point, saturation magnetisation, coercive force) change, but though the changes cause a decrease in the intensity of magnetisation, the direction is preserved.

We have attempted to duplicate the process of oxidation in the laboratory. In our experiments thin disks (2.5 cm in diameter, by 1.5 mm thick) of basalt were cut from cores drilled into the pillows. Each slice was progressively demagnetised in an alternating field up to a peak of 1,000 oersted. Next, the sample was kept in a field 1.0 of gauss at 150 °C in water at typical sea-floor pressures (~0.5 kbar) for a length of time, then cooled to room temperature in the 1.0 gauss field, and finally the thermoremanent magnetisation (TRM) was demagnetised in an alternating field (a.f.). This cycle was repeated with increasing time intervals until over 3,000 h at 150 °C had been accumulated. Two experiments were carried out on pillows dredged from the Median Valley of the Mid-Atlantic Ridge at 45 °N.

The radial variation of the composition of the titanomagnetites was measured³. By taking the lowest Curie temperature phase as stoichiometric titanomagnetite an x-value of 0.6 is obtained. If it is assumed that x remains constant throughout, then z can be obtained from the Curie temperature.

The a.f. demagnetisation curves for the partial thermal remanences (PTRMs) acquired by the first sample (56-3-35—the 35 indicating a sample 35 mm from the pillow rim), which had

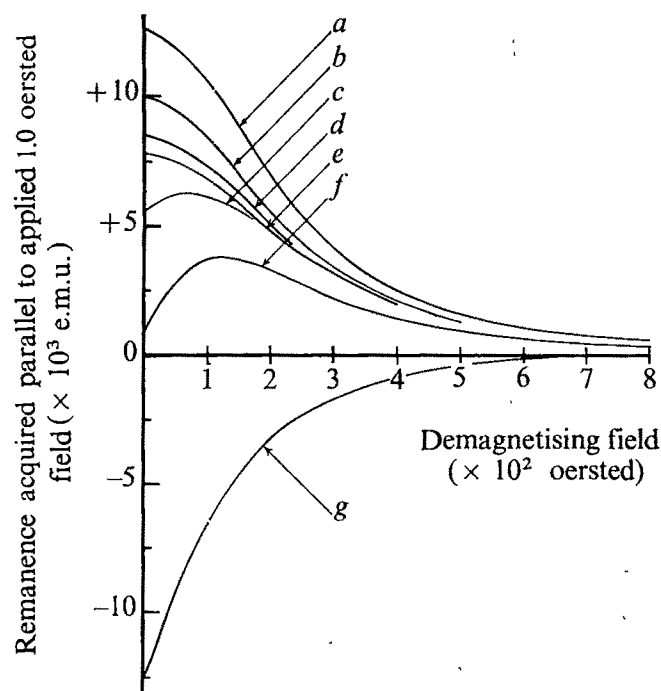


Fig. 1 Alternating field demagnetisation curves for successive PTRMs acquired by sample 56-3-35 on cooling from 150 °C in a 1.0-oersted field. Cumulative times at 150 °C: a, 46 h; b, 174 h; c, 1,070 h; d, 3,545 h; e, 588 h; f, 3,370 h; g, 3,110 h.

an initial $z \approx 0.35$, are shown in Fig. 1. First there was a long interval during which PTRM acquisition slowly decreased with a corresponding slight increase in hardness, seen by normalising the curves. Next the sample started to acquire a component of remanence anti parallel to the external field, until after 3,110 h it had reversed completely. After further heating the PTRM reverted to normal and became indistinguishable from PTRM acquired at much shorter times. This sort of transient self reversal is similar to that described by Ozima and Ozima⁵.

In the second experiment one sample showed complete self reversal; two showed very small reverse components and two showed no reversal at all. The a.f. demagnetisations of the PTRMs for the self-reversed sample (197-8-45), with initial value of $z \approx 0.15$, are shown in Fig. 2. The final curve in this case clearly represents an intermediate state between the 1,070 and 3,110 h states for sample 56-3-35. Curie point measurements at successive stages of alteration for this sample showed an increase in 'z' to about 0.7 followed by splitting of the cation-deficient titanomagnetite so that a higher Curie point (~450 °C) phase is identifiable as well as a phase with the initial Curie point. Self reversal can be shown to accompany the phase splitting in some samples. An attempt to speed the reaction by heating at 210 °C caused phase splitting but no self reversal.

We suggest that the self reversal is caused by an interaction between the titanium-poor daughter phase with the Curie point at 450 °C and the original titanomagnetite. Domains of daughter phase will acquire a chemical remanent magnetisation (CRM) parallel to the external field as they exsolve from the parent. This will occur as they grow in size during the time spent at 150 °C, increasing their relaxation time, until they are normally magnetised, stable, single domains⁶. On cooling, the previously a.f. demagnetised, mother phase will be subjected to both the external field and an interaction field from the daughter phase with which it is in contact. It is conceivable that the interaction field will dominate and thus the mother phase will acquire a reverse PTRM. It is possible that the reverse PTRM of the mother phase will cause a net reverse moment in the sample^{6,7}.

It is not possible to decide definitely whether this is a magnetostatic or an exchange interaction. Experiments with stronger

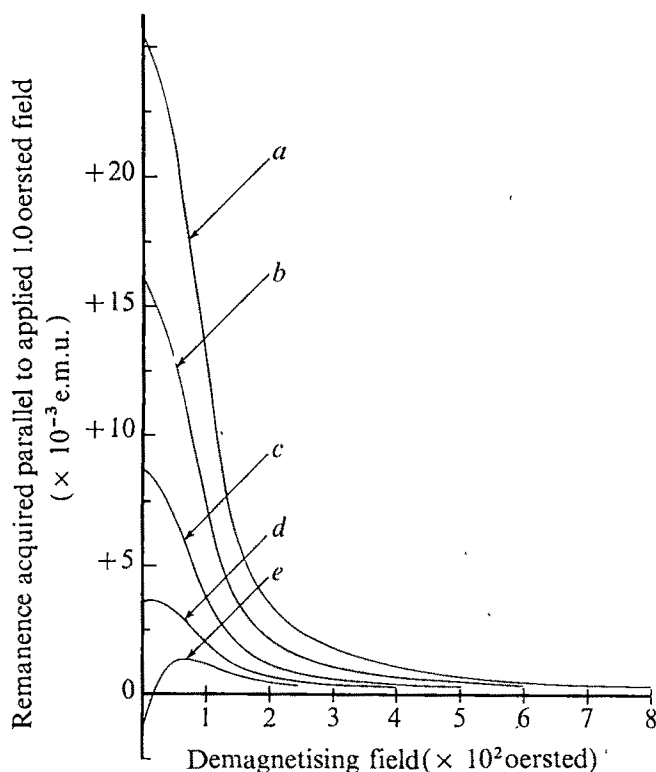


Fig. 2 Alternating field demagnetisation curves for successive PTRMs acquired by sample 197-8-45 on cooling from 150 °C in a 1.0-oersted field. Cumulative times at 150 °C: a, 82 h; b, 166 h; c, 622 h; d, 2,351 h; e, 3,023 h.

fields may be able to resolve this question. The reverse PTRMs are, however, softer (mean demagnetisation field = 80 oersted; compare with 200 oersted; MDF = 80 oersted; compare with 140 oersted for the two samples) than the normal PTRMs. It has been found^{8,9} that the reverse TRM caused by exchange interaction is harder than the normal TRM, so perhaps the present case is an example of magnetostatic interaction.

An important question is whether we should anticipate self-reversed basalts in the ocean crust. In nature the daughter regions could acquire a reverse CRM as they are exsolved in the interaction field of the mother regions. A necessary condition for self reversal is the splitting of the original titanomagnetite into two or more phases. During the phase splitting, conditions exist which may lead to self reversal. Phase splitting of titanomagnetites caused by oxidation on the sea floor at very low temperatures has not been observed. It is, however, well known from laboratory¹⁰ and field studies¹¹ that alteration at 100 °C to 150 °C causes phase splitting in basaltic titanomagnetites.

These experiments suggest that though self-reversed rocks may not be found on the sea floor, they may occur wherever the temperature has been sufficiently raised to split the cation-deficient titanomagnetite into two or more phases, perhaps at depth in the oceanic crust and perhaps in continental regions of hydrothermal alteration.

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Sliding friction of rubber

WE report experiments demonstrating that it is possible to predict quantitatively the level of friction arising when a smooth rubber sphere slides on glass. Analysis of the sliding friction is based on a surface energy approach. Observations show that when a smooth rubber surface advances over glass by a continuous peel process (Schallamach waves) the work done in the region of contact can be calculated in terms of a rate-dependent surface energy and thus an expression found for the tangential frictional stress required to maintain uniform relative motion between the surfaces. In some cases predicted and observed frictional stresses agree to within 10%.

Schallamach observed¹ that for sliding of soft rubber spheres on hard tracks relative motion between them was often only due to "waves of detachment" crossing the region

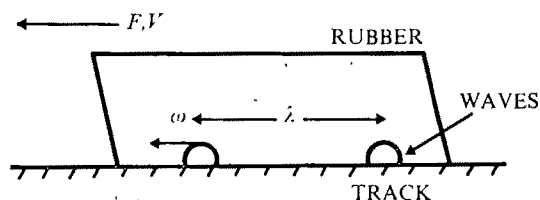


Fig. 1 Generation of Schallamach waves at rubber-track interface $F = \gamma\omega/\lambda V$.

of contact. Between these waves there was strong adhesion. The waves moved as folds in the rubber surface and all gross displacement seemed to be solely associated with them. More recent observations² have revealed some conditions for their appearance. The waves have been thought to originate from elastic^{2,3} or viscoelastic² instability due to tangential stresses in the contact region, so causing the rubber surface to buckle. Before the rubber surface can buckle to form a wave, however, it must first be unpeeled from the hard track⁴. To unpeel smooth soft rubber from glass requires considerable energy^{4,5} and the process is greatly influenced by the viscoelastic behaviour of the rubber^{6,7}. There is evidence that the apparent surface energy required to peel apart these surfaces is of the form $\gamma = \gamma_0 f$ (hysteresis) where γ_0 is the equilibrium energy associated

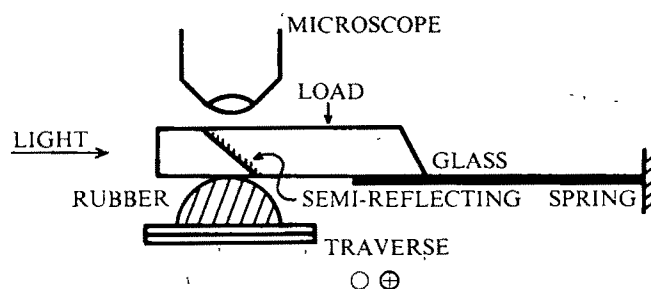


Fig. 2 Apparatus to investigate the role of Schallamach waves in sliding rubber friction. The glass plate is a prism beamsplitter designed for viewing the contact region between rubber and glass in high optical contrast⁸. Rubber hemispheres of various vulcanisates were moulded to give an optically smooth surface¹⁰ of radius 1.85 cm. A variable speed electric motor drove a traverse to move a hemisphere back and forth (\odot/\otimes perpendicular to plane of paper) under the glass plate.

Table 1 Wave observations and the friction of rubber hemispheres on glass

Rubber vulcanisate (Hardness IRHD)	Body velocity V ($\mu\text{m s}^{-1}$)	Centre of contact observations			Frictional stress (MPa)		
		ω (mm s^{-1})	λ (mm)	γ (J m^{-2})	Central	Theory Integrated	Observed
Natural rubber							
peroxide cure (35)	590	17.4 ± 4	1.3 ± 0.2	5.98	0.14	0.16 ± 0.03	0.15 ± 0.02
sulphur cure (44)	240	5.9 ± 0.6	0.34 ± 0.1	5.01	0.36	0.53 ± 0.015	0.48 ± 0.04
Polychloroprene (48)	100	2.3 ± 0.5	0.62 ± 0.1	21.4	0.79	0.83 ± 0.07	0.70 ± 0.03
Styrenebutadiene (50)	110	2.9 ± 0.3	0.52 ± 0.08	9.12	0.46	0.44 ± 0.02	0.45 ± 0.03
Butyl rubber (34)	50	0.59 ± 0.06	1.2 ± 0.2	15.1	0.15	0.16 ± 0.03	0.20 ± 0.03
Polybutadiene (53)	110	4.0 ± 0.8	0.34 ± 0.08	1.78	0.19	0.20 ± 0.03	0.26 ± 0.03
Acrylonitrilebutadiene (43)	30	0.70 ± 0.2	0.6 ± 0.1	5.76	0.22	0.22 ± 0.06	0.29 ± 0.02

with the change in interfacial area and where the function of hysteresis may be up to 10^4 in magnitude⁸. Thus the question arose as to whether the unpeeling of rubber from the track was the main origin of the frictional work that is done in sliding the sample⁴.

The idea of a rate-dependent surface energy has been applied to Schallamach waves in the following way⁴. Consider a rubber slab pulled at a velocity V over a hard track by a tangential stress F (Fig. 1). The waves move with a velocity ω and their spacing apart is λ . If γ is the rate-dependent surface energy per unit area required to peel the rubber from the track and if the energy dissipation in the bulk of the rubber is neglected, then in steady-state sliding where the elastic energy of the rubber is not varying the loss of energy is solely associated with the

peeling. In time dt the stress F does work $FVdt$ per unit area and the energy lost by waves is $(\gamma\omega/\lambda)dt$. Equating these gives

$$F = \gamma\omega/\lambda V \quad (1)$$

Some energy is returned each time surfaces readhere in the wake of a Schallamach wave, but this is negligible⁴ compared to that lost in peeling. The validity of equation (1) was assessed approximately by using the published wave observations of others^{1,2} together with estimates of γ at the relevant rates of peeling ω and it has been shown⁴ to predict the correct order of magnitude for the sliding frictional force of a smooth rubber contact.

To investigate more directly the relation between surface energy and rubber sliding friction we have constructed apparatus to measure simultaneously frictional stress, wave velocity and wave spacing for the sliding at uniform velocity of a smooth spherical rubber surface over a polished glass plate (Fig. 2). The plate normally loaded on the rubber was attached to a supporting spring, tangential deflections of which gave a measure of the frictional force between the sliding surfaces. The sliding contact area was viewed through a low power microscope and by using a double light flash technique it was possible from a single photograph to deduce the wave velocity ω and spacing λ (Fig. 3). Any wave passing through the contact was photographed twice and from the displacement, knowing the time interval between flashes, its velocity ω could be found. Measurement of the distance between waves of the same flash set gives λ . The surface energy γ is rate dependent and was determined from independent sets of experiments; measurement of the contact radius between a smooth sphere of rubber and a glass plate with no applied load yielded a value for the surface energy required to separate the surfaces¹¹, the rate at which the surfaces were separated determined the magnitude of γ for a particular vulcanisate⁴. Thus for a range of vulcanisates rate-dependent γ values were found. At the peel rates set by the Schallamach waves, γ values are as much as two or three orders of magnitude greater than the equilibrium surface energy γ_0 generally found for hydrocarbon surfaces.

The experimental observations are shown in Table 1. If average values of ω and λ are taken midway across the contact, agreement between theory and experiment is good. The Schallamach waves, however, do not travel at a uniform velocity². From photographs such as Fig. 3, it was possible to measure accurately the variation in velocity ω and spacing λ of the waves as they crossed the contact area. Point values of the frictional stress F were calculated using equation (1) and then summed across the contact to arrive at a more accurate theoretical value of the total frictional stress. In some cases the integrated theoretical frictional stress is within 10% of the direct observations (Table 1).

Thus it seems that for a wide range of vulcanisates, at least in the case of smooth surfaces, surface energy of peeling probably has a central role in the frictional behaviour of rubber. Furthermore, this picture gives a ready mechanism for the known viscoelastic behaviour of rubber friction because the peeling process has already been established as being viscoelastic in character. This is believed to be the first time such an



Fig. 3 Typical double flash photograph of Schallamach waves for natural rubber. The duration of each flash was $1/1,200$ s and the interval between flashes could be varied between $1/100$ s up to 1 s using a simple delay system. To distinguish clearly between each set of waves one light flash was arranged to be slightly less intense than the other so that on a black and white photograph one wave set appears darker than the other. The major axis diameter of the elliptical contact area in this photograph was 3.2 mm at a sliding velocity of $570 \mu\text{m s}^{-1}$ under a normal load of 0.085 kg.

approach has been taken to provide a quantitative description of rubber friction based on independently measurable physical quantities. Such a picture avoids having to invoke any specific structure or molecular jump theory.

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Relaxation of the Bernal model

RANDOM packings of spheres serve as models for a variety of noncrystalline particulate and molecular aggregates, some of which, for example the amorphous phases of metals and alloys, offer abundant possibilities for the comparison of theory and experiment^{1–4}. Nevertheless there remain significant discrepancies between the measured structural characteristics of real systems and the geometrical models considered by Bernal⁵, Cohen and Turnbull⁶ and others to be prototypes for the simple vitreous state. The most enduring of these is perhaps the difference in position and intensity between the two components of the split second peak in the radial distribution function observed in actual and artificial systems^{2–4}. Attempts to remove this disagreement by variation of the algorithm used in computer-simulated packings have only been partially successful. In particular Sadoc *et al.*² have noted that it is relatively easy to build in strong local density inhomogeneities associated with icosahedral arrangements^{7,8} and Connell has experimented with the packing of compressible spheres, showing that significant shifts in the radial distribution function result from the softening of the interatomic potential⁹.

We thought it worthwhile to 'relax' the '1968' (ref. 10) Bernal sphere-packing model under a realistic interatomic potential function to observe which of the resultant geometrical changes can occur fairly easily in an initially dense packing, as distinct

Table 1 Frequency of polyhedron types

	Hard sphere	Relaxed	Crystal close to melting point†
'Crystalline'			
(0 3 6)*	0.32	0.34	0.40
(0 4 4)	0.22	0.27	0.44
(0 5 2)	0.06	0.03	0.07
	0.60	0.64	0.91
'Non-crystalline'			
(0 0 12)	0.02	0.04	<0.01
(0 1 10)	0.08	0.11	0.01
(0 2 8)	0.14	0.17	0.06
	0.24	0.32	0.07

Only clearly significant types are shown. Hence the proportions given do not total to unity.

*The polyhedron type ($n_3 n_4 n_5$) is characterised by n_3 , n_4 , n_5 faces with three, four and five edges, respectively.

†This column refers to a Monte Carlo simulation of crystalline argon at 84 K using a Lennard-Jones potential function¹⁰.

from those which may need to be induced in the building process. We used quite standard computational techniques for the 'optimisation' of many-variable potential functions¹¹, applying a Lennard-Jones 6:12 potential between all atoms present and allowing the program to seek out a natural potential energy minimum in the neighbourhood of the initial 'Bernal' configuration. Even with the best programs available, however, we could not have hoped to handle the whole 7,934-atom Bernal packing and so were obliged to extract a central core of some 999 atoms for the calculation.

The algorithm used was based on a version of the Fletcher-Reeves conjugate-gradient method¹², supplied to us by the Theoretical Physics Division, AERE, Harwell, and run on the London University CDC 7600 machine. We took precautions to see that the program searched thoroughly in the near vicinity of the original point in configuration space, avoiding as far as possible the unnatural rearrangement of atomic positions which might have occurred with a 'dynamic' optimisation method. Nevertheless, the final configuration obtained cannot be expected to be in any way unique and there will undoubtedly be a multitude of alternative minima at similar or smaller distances, from the 'Bernal' point on our 2,991-dimensional energy surface. We assume only that the short-range geometrical correlations at the minimum found shall be statistically representative of the whole class of alternatives which might be discovered by repeated excursions in the neighbourhood of the 'Bernal' configuration.

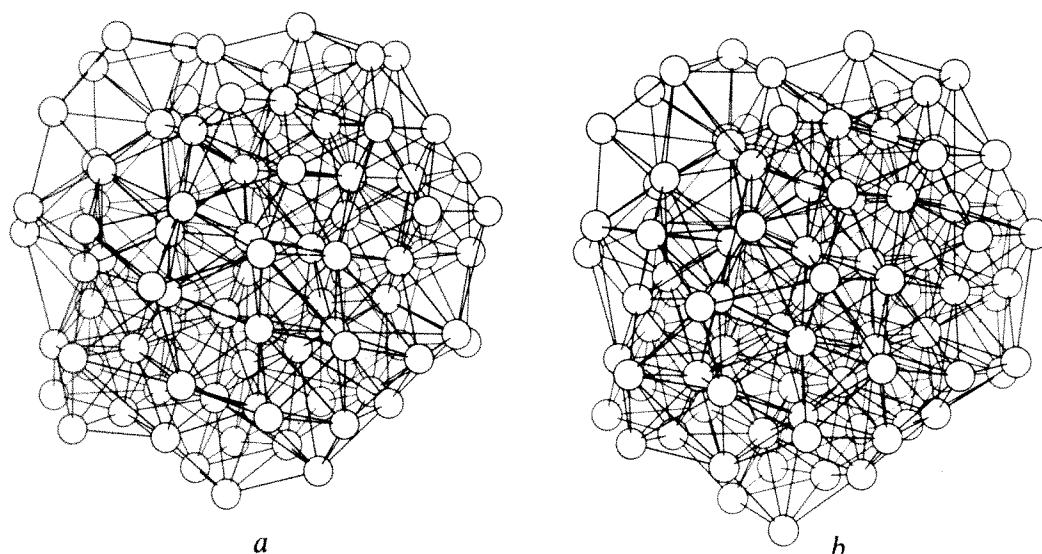


Fig. 1 The Bernal model before (a) and after (b) relaxation under the Lennard-Jones potential. Only the 100-atom core of the 999-atom assembly is depicted. Atoms within a distance $\sqrt{2}$ of each other are shown bonded. The radius of the spheres is drawn at 0.15 of the diatomic equilibrium distance.

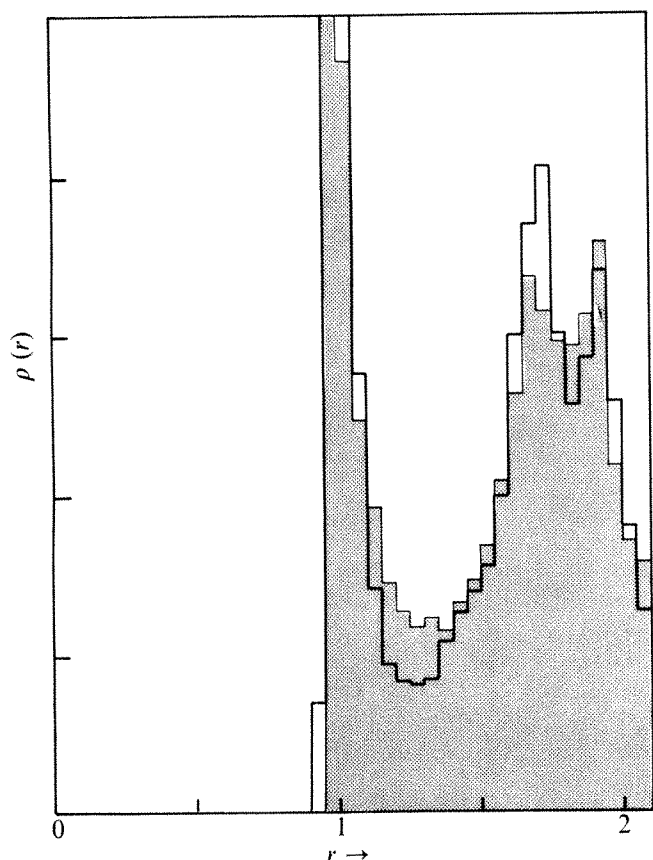


Fig. 2 Radial distribution function for the relaxed and unrelaxed Bernal model. The histogram for the original system (shaded) is superimposed on that for the relaxed (blank). Distance in terms of the Lennard-Jones pair distance. Vertical scale in arbitrary units.

The results of this exercise can be presented in various ways. Fig. 1 shows computer-generated drawings of the central core of 100 atoms extracted from the 999-atom aggregate before and after relaxation. We were able to scrutinise many such drawings at different angles and in stereoscopic form to confirm the impression given by the figure that perceptible changes have taken place in the spatial relationship of neighbouring atoms even though, for the most part, the topology of nearest-neighbour connections is relatively undisturbed.

The resulting structural changes in fact gave rise to a corresponding decrease in total potential energy of some 5.1% from the Bernal configuration. This, we estimate, would have decreased further by some 0.3% had we continued computing for quite unjustified times. The r.m.s. distance moved by the spheres was also monitored and amounted to approximately one-fifth of the nearest-neighbour distance averaged over the whole assembly. Detailed examination of the coordinates showed that a disproportionate contribution to this figure came from atoms at or near the surface of the model which underwent relatively large adjustments.

Figure 2 shows the averaged radial distribution function of the 999-atom model before and after relaxation, computed using the Mason correction¹³. As expected, the first peak becomes less sharp while the first minimum is deepened. More significantly, the two components of the split second peak reverse in intensity in precisely the manner observed in amorphous Ni-P and other disordered alloys²⁻⁴. There is also a suggestion of a splitting in the smaller third peak. Otherwise the radial distribution function is unexceptional and the peak positions, which in general can be expected to depend somewhat on the nature of the potential, remain virtually unchanged for the Lennard-Jones case.

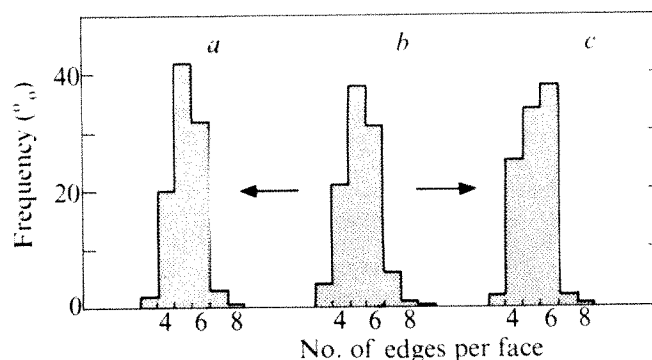
A more sensitive indication of the changes in local structure is to be found in the statistics of the Voronoi polyhedra¹⁵ before and after relaxation. Table 1 shows the frequency of some particularly significant polyhedron types for the random packing before and after relaxation, in comparison with those for the crystal near the melting point. There are significant changes in the statistics of faces per polyhedron which together lead to a reduction in the average number of faces \bar{N} from 14.20 in the Bernal configuration to some 13.99 in the relaxed form. (Compare values of 14.46 and 14.07 for Lennard-Jones liquid and solid phases, respectively, at the melting point¹⁰.) On more detailed analysis we observed the following:

- (1) The size distribution of normalised polyhedron volumes V_1/\bar{V} indicating the variation of local volumes effectively occupied by atoms shows relatively slight adjustment on relaxation. A slight downward shift in the maximum is observed with only small change in standard deviation (0.039 and 0.037, respectively, before and after). This indicates that the overall volume decrease in the packing goes with a redistribution of local volumes rather than simply a rescaling.
- (2) The frequency of the five-edge faces increases at the expense of others, particularly the three- and seven-face components, but otherwise the qualitative difference between 'random' and 'crystalline' polyhedron statistics is maintained (Fig. 3).
- (3) On analysing the statistics of occurrence of actual polyhedron types rather than of faces, significant trends may be distinguished. Thus the occurrence of the (0 0 12) polyhedron, indicative of distorted icosahedral arrangements, shows an appreciable increase in the relaxed array (Table 1). This trend is also shared by the (0 1 10) and (0 2 8) types which also arise from the distorted icosahedron. Crystallisation must result in a large increase in the occurrence of (0 4 4) and (0 3 6) types; these contribute little in the original packing and show no particular increase in relaxation.

The most striking single feature of these results is undoubtedly the fall in the average face-count \bar{N} on relaxation. In view of (1) to (3) and the lack of any visual evidence in the drawings, this cannot be attributed to incipient crystallisation and we conclude that we have observed a spontaneous reinforcement of locally 'tetrahedral' order⁹⁻¹¹, perhaps occasioned by the formation of distorted motifs related to the 13-atom icosahedron. That these do not seem to have occurred on the scale predicted in the experiments of Sadoc *et al.* is not surprising in as much as we have taken as our initial configuration the particularly dense arrangement based on the Bernal ball-bearing model. Even with the soft potential used, this may have introduced unnatural constraints, with atoms 'jammed' and unable to take up more efficient minimum configurations without passing by way of complicated high-dimensional saddle points that are inaccessible with the program used.

How worthwhile it may be to elaborate further packing structures without reference to the physical deposition process

Fig. 3 Comparison of the Voronoi polyhedron face-frequency for: a, the relaxed Bernal model; b, the hard-sphere Bernal model; c, the Lennard-Jones crystal at melting point.



it is desired to model seems debatable. There may simply not be a single canonical structure appropriate to vitreous packings generated in unspecified experimental conditions. In any case it seems necessary to assert that, in view of the above results, the Bernal-Cohen-Turnbull view of the random close-packed structure as one essentially determined by hard-sphere repulsions and a particular, limited set of basic polyhedral relationships may need refinement when applied to the aggregation of real atoms under definite thermodynamic and mechanical constraints.

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Examination of ancient pottery using the scanning electron microscope

A SEQUENCE of pottery sherds from Iraq spanning the period about 6000 BC to 750 AD and two sherds from Turkey about 5000 BC have been examined using a scanning electron microscope (SEM). This examination provided information on the internal morphology developed during firing and in particular information on the extent of vitrification (the glassy phase) and the pore structure^{1,2}. The extent of vitrification provided a useful parameter for characterising the quality of the pottery since it influences several physical properties (such as, hardness, strength, permeability) which are relevant to its suitability for the various uses to which it might be put. In addition by refiring samples of the pottery at known temperatures and determining, by re-examination with the SEM, the temperature at which an increase in the vitrification had occurred, it was possible to estimate the firing temperature used in antiquity.

Fresh fracture surfaces in the pottery, coated with a thin layer of Au-Pd, were examined in a SEM (Cambridge S600) both before and after refiring at known temperatures in the range 750 to 1,250 °C. The refirings were carried out in a tubular furnace in air at a heating rate of 200 °C h⁻¹ with a soaking time of 1 h at the peak temperature.

The nature of the internal morphology observed with the SEM together with X-ray powder diffraction data established that all the pottery was manufactured from calcareous clays. On the basis of the stage which had been reached in the progressive development of vitrification, it was possible to divide the pottery into four groups (Table 1). The first group (NV) contains pottery in which there is no vitrification and the

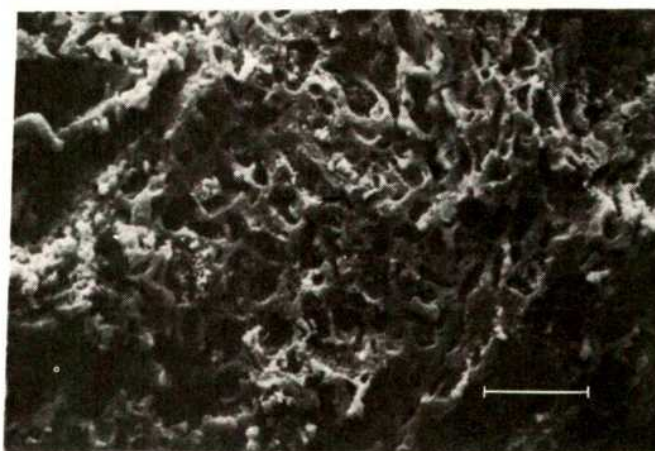


Fig. 1 Scanning electron micrograph of extensively vitrified pottery sherd (V) from Nineveh (Neo-Assyrian), Iraq. Bar represents 10 µm.

aggregates of flaky clay particles are essentially the same as in raw clay. The second group (V) exhibits extensive vitrification with a network of smooth-surfaced glass filaments forming an open or cellular structure over the fracture surface (Fig. 1). In the third group (V+), the cellular structure has begun to coarsen and larger areas of glass are present. Finally the fourth group (TV) exhibits total vitrification, the cellular structure having disappeared and been replaced by a continuous vitrified surface containing isolated pores (Fig. 2).

By means of refiring experiments on the pottery, together with the data obtained from examining specimens of calcareous clays fired at known temperatures and showing a similar pattern of vitrification development, it was possible to assign approximate firing temperature ranges to the various vitrification stages as indicated in Table 1. In assigning these ranges, it was assumed that the firing times (heating plus soaking time) employed in manufacture were comparable with those employed during refiring. Significantly faster heating rates (say 800 °C h⁻¹) and shorter soaking times (5 min) would have required firing temperatures higher by about 50 °C to achieve the same extent of vitrification. Second, on the basis of the colour of the pottery, it was assumed that it had been fired in an oxidising atmosphere. If fired in a reducing atmosphere, firing temperatures lower by about 50 °C would have produced an equivalent vitrification.

The pottery from Iraq was all fired to sufficiently high temperatures to produce, at least, extensive vitrification (V)

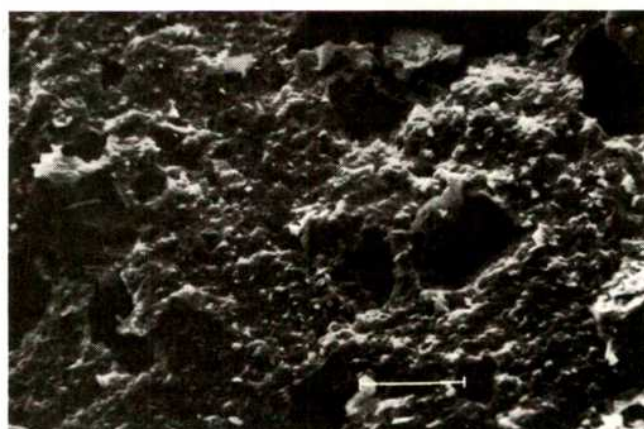


Fig. 2 Scanning electron micrograph of totally vitrified pottery sherd (TV) from Ubaid, Iraq. Bar represents 10 µm.

Table 1 Vittrification stage and firing temperature of ancient pottery

Provenance*: Period	Approximate time ranges	Number of sherds			
		NV < 800 °C	V 850–1050 °C	V+ 1050–1150 °C	TV > 1150 °C
Choga Mami (Jarmo)	c. 6000 BC		1		
Samarra	c. 5500–5000 BC		2	1	
Arpachiyah (Halaf)	c. 5000–4500 BC		4		
Ubaid, Ur, Eridu (Ubaid)	c. 4500–4000 BC		1	2	4
Nineveh: IV/V	c. 3500–2800 BC		3	1	1
Jemdet Nasr	c. 3500–3000 BC		1	1	
Kish: Early Dynastic	c. 2900–2400 BC		3		
Kish: Old Babylonian	c. 1800 BC		1		
Nineveh: Neo-Assyrian	c. 800–600 BC		2		
Nineveh, Kish: Parthian	c. 150 BC–250 AD		3		
Kish: Sasanian	c. 250–650 AD		1		
Hacilar	c. 5000 BC	2			

*Type-site given in brackets where this differs from provenance. Except for Hacilar, which is in Turkey, all the sites are in Iraq.

and for most of the periods under consideration, this group predominates (Table 1). The principal exception is the Ubaid pottery which exhibits chiefly total vitrification (V+ and TV), indicating that consistently higher firing temperatures were employed during this period. It is therefore apparent that the technology required to manufacture extensively vitrified pottery using calcareous clays was developed in Iraq at a very early stage in the production of pottery and that this tradition in ceramic technology remained essentially unchanged over a 6,000-yr period. Furthermore the tradition in Iraq contrasts with that in Turkey during the period around 5000 BC where, although calcareous clays were again used, the firing temperatures employed were significantly lower and the pottery produced exhibits no vitrification (NV).

The use of calcareous clays, although obviously determined in part by their availability, is of interest since this type of clay possesses distinctive vitrification properties which facilitate firing². First, extensive vitrification (V) can be achieved at the comparatively low firing temperature of about 850 °C. Second, the characteristic cellular structure associated with extensive vitrification remains essentially unchanged over a temperature range of about 200 °C (from 850 to 1,050 °C) and therefore the control of the temperature attained during firing is not particularly critical. This contrasts with the situation for non-calcareous clays where the extent of vitrification increases progressively with increasing temperature so that the quality of the pottery produced can vary quite considerably with changes in firing temperature of about 50 °C. But in the context of estimating the firing temperatures employed in antiquity, the thermally stable structure associated with calcareous clays results in a wide temperature range being assigned to the extensive vitrification group (V) in Table 1.

These results establish that, because information is obtained on both the extent of vitrification and the firing temperature, the examination of ancient pottery with an SEM is valuable for characterising and distinguishing between the different traditions in ceramic technology in antiquity. Since the firing temperature required to produce a particular degree of vitrification can vary by as much as 200 °C, depending on the refractoriness of the clay and the firing atmosphere used, either the extent of vitrification or the firing temperature by itself does not fully characterise the ceramic technology. For example, the same extent of vitrification can be achieved by firing a calcareous clay at a low temperature (850 °C) or a refractory non-calcareous clay at a higher temperature (950–1,000 °C). Taken together, however, the extent of vitrification and the firing temperature reflect the technological capabilities of the ancient potters with respect to both their ability to achieve the necessary temperatures and their understanding of the refractory properties of the clay used. In this respect, therefore, the examination of ancient pottery with an SEM is more valuable than an estimate of the firing temperature by itself,

such as can be obtained either by studying those minerals altered during firing^{3,4} or by thermal expansion measurements⁵.

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Temperature-independent relaxation in a lamellar block copolymer

It has long been assumed that all processes giving rise to stress relaxation in high polymers are very dependent on temperature. The peak in mechanical or dielectric loss factor ($\tan\delta$) can be used to characterise the most probable relaxation time of a high polymer solid. The temperature locus of this relaxation time is described by a simple Arrhenius-type law for low temperature, glassy state processes¹ and by the Williams, Landel and Ferry expression² for ' T_g ' processes. We now report results for an elastomer exhibiting a unique relaxation process which remains essentially constant in position and magnitude over a temperature range of more than 100 K.

The block copolymer used was a poly(α -methylstyrene-*b*-dimethylsiloxane) prepared with 'star' geometry $(AB)_4X$, where A represents a poly(α -methylstyrene) block, B a poly(dimethylsiloxane) block and X is a tetrafunctional coupling atom. This polymer was synthesised by Dr D. Jones, Dow Corning Company, by sequential anionic copolymerisation³. The product contained 37.5% (w/w) poly(α -methylstyrene), molecular weight 9,000, with the molecular weight of the B block 15,000. In common with other block copolymers, different morphologies can be achieved by differing the physical treatment⁴. In the present case lamellar morphology could be generated by compression moulding a sheet (523 K, 13 MPa (130 bar)) with considerable flow in the plane of the sheet or by slow casting from benzene on a mercury surface. Dynamic mechanical measurements were carried out either in bending geometry (bars $\approx 20 \times 10 \times 2$ mm) to give Young's modulus (E') or in shear sandwich geometry (cubes with side 2 mm) to give the rigidity over wide ranges of temperature and frequency on a slightly modified version of a phase- and amplitude-measuring mechanical spectrometer previously reported^{5,6}.

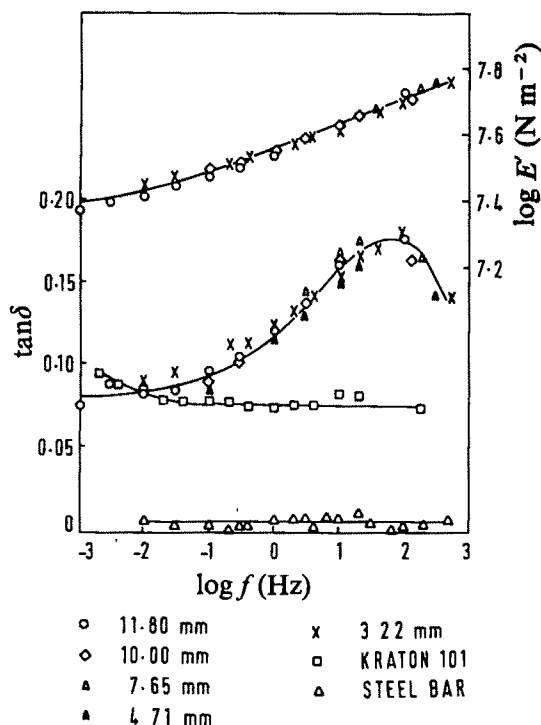


Fig. 1 Frequency dependence of mechanical loss factor ($\tan\delta$) and Young's modulus (E') for a benzene-cast sample (294 K) in the bending mode with different clamped lengths as indicated.

Results are shown in Fig. 1 for the benzene-cast sample in a bending mode perpendicular to the sheet direction at 294 K. A clear mechanical loss peak occurs at about 90 Hz and the data remain constant with temperature as shown in Fig. 2. Proper working of the apparatus was checked using standard samples (a high tensile steel bar and a Kraton 101 (Shell) block copolymer) with the results reported in Fig. 1; no abnormalities were evident. Further checks were made by measuring five bars of different lengths of the copolymer under study. The data all lie on the same curve, indicating the reality of the result and the accuracy of the measurements.

Both methods used to produce the lamellar morphology also cause orientation of the lamellae in the plane of the cast or moulded sheet. Small angle X-ray diffraction has been used to characterise both the lamellar spacing and orientation, using both slit and pinhole optics as described previously⁷. Four orders of diffraction are observed, with the ratio of the derived d spacings 1:0.52:0.335:0.254 (from pinhole optics and photographic detection) for the moulded sample and 1:0.489:0.328:0.242 for the benzene-cast sample giving certain evidence of lamellar structure. Pinhole data were used to establish that uniaxial symmetry exists about the normal to the plane of the sheet and to evaluate the orientation function⁸ $\langle \cos^2\phi \rangle$ where ϕ is the angle between lamellar planes and the perpendicular to the sheet. The orientation function is 0 for perfect orientation and 0.333 for random orientation. The benzene-cast sample gave $\langle \cos^2\phi \rangle = 0.215$ and the more highly orientated, moulded sample $\langle \cos^2\phi \rangle = 0.016$.

The mechanical properties of these oriented systems will clearly be anisotropic and their directional dependence has been studied for the experimentally accessible situations, which will be reported by us in full elsewhere. Cubes were cut from the sheet of the benzene-cast sample and clamped so that shearing occurred on the faces of the sheet (geometry 1) and perpendicular to the sheet (geometry 2). The relaxation processes are shown for the pertinent samples in Fig. 3. The simplest case of shearing in the plane of the lamellae (geometry 1) produces a low modulus (10 MN m⁻²) and also a low magnitude transition, whereas

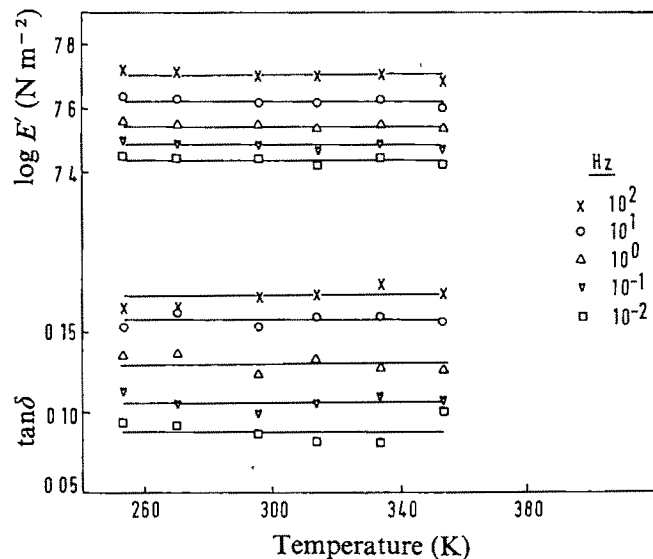
shear perpendicular to their plane (geometry 2) produces a larger loss peak. This indicates that shearing processes alone between lamellae are not responsible for the mechanical loss. In the Young's modulus measurements, the highly oriented, moulded sample exhibits a low relaxation magnitude whereas the corresponding benzene-cast sample with inferior orientation yields a large relaxation magnitude, as does shear with geometry 2. This is good evidence that bending of lamellae is a key feature of the loss process, with lamellae at large angles to the stress direction deforming by bending before undergoing possible rotation and shear.

The results do not conform to the simple interlamellar shear model of Iwayanagi⁹ nor to the more refined model of McCrum and Morris¹⁰, both of which were proposed for semi-crystalline polymers. Two models are now proposed for the relaxation processes: (1) cantilever bending with relaxation by interlamellar shear and rotation, and (2) cantilever bending in a viscous medium. Each is a 'pack of cards' model with sheets of glassy state poly- α -methylstyrene alternating with sheets of lightly cross-linked polydimethylsiloxane. The layers bend under stress with viscous resistance from the accompanying interlamellar shear. The thickness of the poly- α -methylstyrene phase (h) and of the polydimethylsiloxane phase (d) are both small (~ 100 Å) compared with the largest in-plane dimension (l), expected to be ~ 1 μ m. The difference between the models is that in case (1) the lamellae are rigidly constrained at a domain boundary at one end only so that each can bend only as a rigidly clamped cantilever, whereas in case (2) the lamellae are only loosely constrained at one end so that they can rotate and give rise to deformation by interlamellar shear. In model (1) each glassy-state lamella bends as a separate cantilever giving rise to different displacements on the top (extended) and bottom (contracted) surface of each. The glassy lamellae can only bend, therefore, if the viscous silicone phase is simultaneously sheared. Voigt retardation time (case (1)) of this model is

$$\tau_1 = \frac{3\eta l^2}{E'h\pi n}$$

where E' is the in-phase component of the glassy lamellar Young's modulus, η (strictly G''/ω) the viscosity of the silicone rubber phase and n the number of lamellae in the domain. Model (2) allows bending by the above scheme (with retardation time τ_1) but also allows a subsequent relaxation of stress through

Fig. 2 Temperature-independent mechanical loss factor ($\tan\delta$) and Young's modulus (E') for a benzene-cast sample in the bending mode at the frequencies shown.



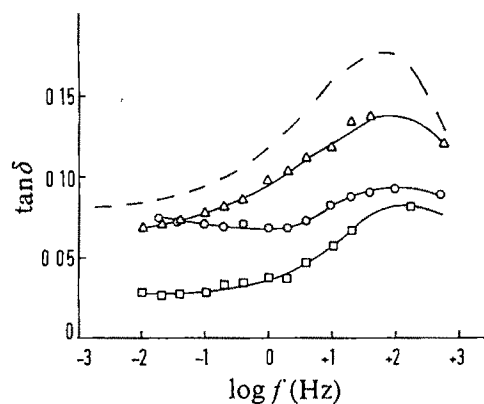


Fig. 3 Mechanical loss factor ($\tan\delta$) in the frequency plane, at 293 K, for moulded (\square) and benzene-cast (—) samples in the bending mode and benzene-cast samples also in shear, in the plane of the sheet (\circ) and normal to the plane of the sheet (\triangle).

interlamellar shear with loss of the bending deformation. This gives rise to a Maxwell model situation with a relaxation time

$$\tau_2 = \frac{3l^4\eta}{5E'dh^3n^2\tan^2\phi}$$

Details of these derivations will be given by us elsewhere. Differences between relaxation and retardation times are fairly trivial because of the low relaxation strengths involved.

Selection of lamellar parameters from SAXS (small angle X-ray scattering) and volume fractions should now allow tests of the relations for τ_1 and τ_2 . For the benzene-cast sample $h = 82 \text{ \AA}$, $d = 153 \text{ \AA}$ and for the moulded sample $h = 98 \text{ \AA}$, $d = 182 \text{ \AA}$. Evaluation of l and n should be feasible by electron microscopy, but sectioning problems have so far made this intractable. From electron micrographs of similar lamellae-forming block copolymers¹¹⁻¹³ and the sharpness of the SAXS scattering peaks, we expect $l \sim 1-10 \text{ \mu m}$ and $n = 30-300$ per domain. E' for poly- α -methylstyrene has been obtained as $2.5 \times$ shear moduli values of Heijboer¹⁴ and η as $E''/3\omega$ from the data of Langley and Ferry¹⁵. We note that $(G''/\omega) = 2.3 \times 10^3 \text{ N s m}^{-2}$ at $\omega = 1$, but decreases to 7.3 N s m^{-2} at $\omega = 1,000$. We will select an approximate value of 10^3 N s m^{-2} corresponding to the centre of the present frequency range but strictly this parameter should be adjusted with frequency. Using the upper estimates for l and n we obtain frequencies of maximum damping $f_1 = 90 \text{ Hz}$ and $f_2 = 4 \text{ Hz}$. The plateau of loss at low frequencies can thus be understood as arising from a range of relaxation times from model (2) which depends on orientation. Domains with lamellae parallel to the stress direction will have $\langle \tan^2\phi \rangle \rightarrow \infty$ and $\tau_2 \rightarrow 0$ and vice versa. The peak in the data at 80–100 Hz is well predicted by the f_1 value above.

Further evidence in favour of this interpretation has been obtained by swelling the block copolymer in silicone fluid of molecular weight 2,000 and viscosity $5 \times 10^{-3} \text{ N s m}^{-2}$. The only effect of this is to increase the silicone polymer thickness d , to decrease its dynamic viscosity and to change the domain orientations to a more random situation (we will take $\phi = 45^\circ$). SAXS showed that, within experimental error, d increased as predicted from unidirectional swelling (16.3%) of the silicone polymer, giving an observed value of d of 180 \AA (an increase of 17.6%). The viscosity of the final silicone phase (140 N s m^{-2}) was evaluated assuming that each fluid obeys the Doolittle equation $\ln\eta = A - B(1/f)$ with constant coefficients and simple additivity of free volume. The damping maxima (swollen system) are predicted to be at 800 Hz and 8 Hz. The experimental data in Fig. 4 give encouraging agreement, with

two clear regions of loss now apparent at $f_1 > 100 \text{ Hz}$ and $f_2 \sim 1 \text{ Hz}$.

The temperature independence of the loss processes in the bulk samples can now be recognised as a fortuitous constancy

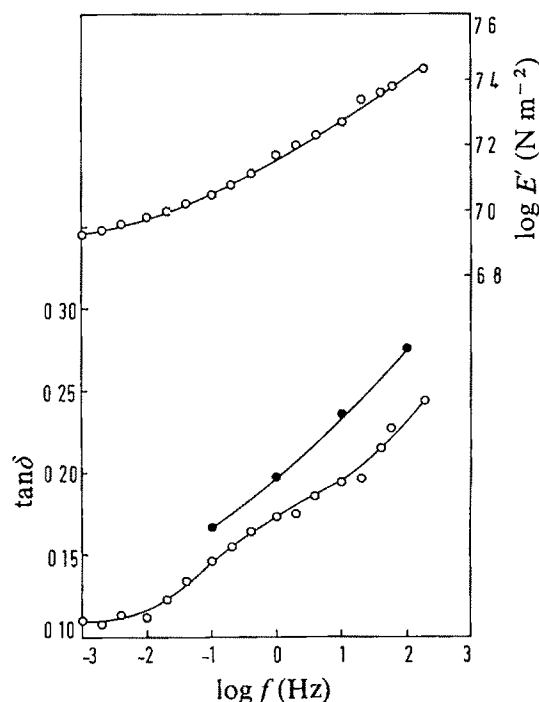


Fig. 4 Mechanical loss factor ($\tan\delta$) and Young's modulus (E') in the frequency plane at 293 K, for a benzene sample swollen with silicone oil. Loss data at 230 K (\bullet) now shows some temperature dependence.

in the ratio $\eta(\text{rubber})/E'(\text{glass})$ with temperature. The swollen sample does not exhibit constancy with temperature, because of a more rapid decrease of η with temperature.

The mechanisms for mechanical loss in the copolymers discussed here are expected to give rise to analogous loss processes in other lamellae-forming block copolymers and in crystalline polymers.

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Re-examination of monotony threshold hypothesis in bird song

HARTSHORNE^{1,2} has suggested that a relationship exists between the degree of versatility and continuity in bird song. According to this view, singing which is relatively continuous, with pauses between successive songs relatively short when compared with the length of the song, would tend to be highly monotonous unless successive utterances were variable in pattern.

Hartshorne lent support to his hypothesis by showing a rough correlation of versatility with continuity³. Continuity was calculated by dividing the song length by its cadence (song length + length of following silent interval). A bird with a ratio ranging from 0 to 0.20 was termed a discontinuous singer, 0.20 to 0.50 a semi-continuous singer, and 0.50 to 1.00 a continuous singer. A versatile species was originally defined as one in which "each normal individual... has a repertoire of four or more distinct songs" all or most of which appear "in no particular order" in singing bouts lasting more than 1 or 2 min⁴. A species that is repetitious, "especially if the song is brief and simple, relatively lacking in interval variety of pitch, rhythm, and so on" is non-versatile⁴. Semi-versatile species fall between the two extremes. When a species was categorised simultaneously as discontinuous, semi-continuous, or continuous, and versatile, semi-versatile, or non-versatile in a three by three table, Hartshorne found that most species fell on a diagonal connecting discontinuous-non-versatile and continuous-versatile (Table 1a).

Hartshorne exploited data obtained mainly by ear. Here we present an assessment based principally on information recorded on tape. A literature search resulted in usable data for 39 species of birds, most of which are found in North America. Data for each bird included song length, number of songs in the repertoire and intersong interval, the last being in some cases derived either for the cadence, or from estimates of number of songs per min. Additional data were found for other species—most commonly song length—but species for which all three measures were not available were excluded from consideration.

The definition of song, and therefore song length, may not be consistent from worker to worker, but each worker's own definition of song length was used even though this may introduce some ambiguity in interspecies comparison. Thus for the mockingbird, Wildenthal⁵ noted that "Hartshorne apparently measured the duration of units corresponding to the groups or phrases of the present study, and thus he included silent periods

Table 1 Data compiled by Hartshorne (a) and the data reported here (b) categorised by versatility and continuity

a	Non-continuous	Semi-continuous	Continuous
Versatile	0?	4	9
Semi-versatile	6	24	5
Non-versatile	40	11	1?

b	Non-continuous	Semi-continuous	Continuous
Versatile	10	14	3
Semi-versatile	0	6	0
Non-versatile	2	4	0

between syllables and syllable clusters in the performance time". Wildenthal noted that mockingbirds were only semi-continuous if intersyllable periods were included in the performance time; therefore Table 2 lists a weighted average of mockingbird syllable-pattern length compared with intersyllable pattern length, which gives a percentage performance time within the range Wildenthal reports as comparable to Hartshorne's figures. For other species as well, averaged figures are included when a range of values was given. In the case of five species, repertoire size was listed only as large when the number of patterns or combinations of patterns became too numerous to estimate accurately. The rose-breasted grosbeak for example may change only a few elements in each successive utterance, producing a new combination, or song, each time⁶; it also has unusually long pauses between songs (Fig. 1).

Song length for each species was divided by cadence to produce a percentage performance time. Using Spearman's rank-correlation coefficient our first test compared repertoire size and percentage performance time, which should be significantly correlated if Hartshorne's predictions are valid; however, no significant correlation was found ($r_s = 0.20$, not significant). Even a simple three by three categorisation of these data gave results markedly different from those of Hartshorne (Table 1b). Song length compared with repertoire size also was not significantly correlated ($r_s = -0.15$, not significant).

Clearly these data do not support Hartshorne's hypothesis. Possible differences in the measurement of versatility and continuity between these data and Hartshorne's require comment. By Hartshorne's original criteria, perhaps as many as 27 out of the 39 species examined would qualify as versatile, although

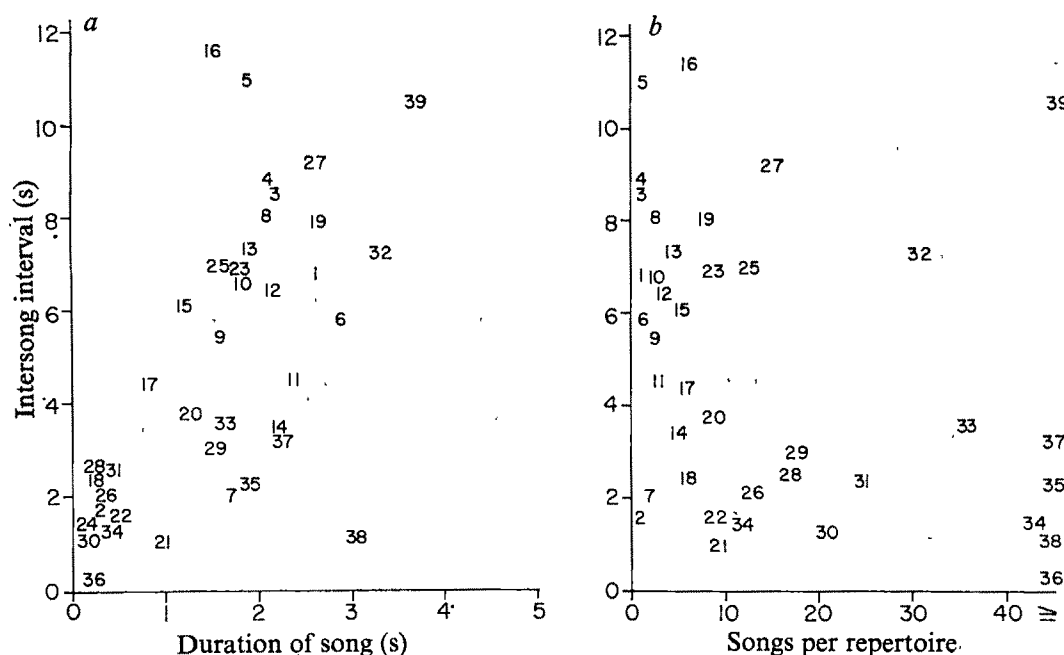


Fig. 1 Correlations of intersong intervals with duration of song (a) and size of repertoire (b). Numbers correspond to species listed in Table 2.

Table 2 Song data for various species of birds

	Species	Repertoire size (songs)	Song length	Intersong interval	Percentage of performance time	Reference
1.	Chipping sparrow (<i>Spizella passerina</i>)	1	2.61	6.79	0.28	10, 11
2.	Huttons vireo (<i>Vireo huttoni</i>)	1	0.29	1.54	0.16	12
3.	Indigo bunting (<i>Passerina cyanea</i>)	1	2.20	8.61	0.20	13
4.	Lazuli bunting (<i>Passerina amoena</i>)	1	2.13	8.79	0.20	13
5.	White-crowned sparrow (<i>Zonotrichia leucophrys</i>)	1+	1.90	11.00	0.15	14
6.	White-throated sparrow (<i>Zonotrichia albicollis</i>)	1+	2.87	5.83	0.33	15
7.	Varied bunting (<i>Passerina versicolor</i>)	2	1.70	2.00	0.46	13
8.	Chaffinch (<i>Fringilla coelebs</i>)	2+	2.10	8.07	0.21	16, 17
9.	Mexican junco (<i>Junco phaeonotus</i>)	2+	1.63	5.46	0.23	18
10.	Veery (<i>Hylocichla fuscescens</i>)	2+	1.84	6.73	0.22	19, 20
11.	Bewick's wren (<i>Thryomanes bewickii</i>)	3	2.39	4.48	0.35	20, 21
12.	Grey-cheeked thrush (<i>Hylocichla minima</i>)	3+	2.14	6.43	0.25	19, 20
13.	Painted bunting (<i>Passerina ciris</i>)	4+	1.91	7.37	0.21	13
14.	Orange-breasted bunting (<i>Passerina leclancheri</i>)	5	2.21	3.40	0.39	13
15.	Happy wren (<i>Thryothorus felix</i>)	5+	1.20	6.10	0.16	(R. N. Brown, unpublished)
16.	Brown towhee (<i>Pipilo fuscus</i>)	6	1.49	11.60	0.11	22
17.	Rufous-sided towhee (<i>Pipilo erythrophthalmus</i>)	6	0.83	4.39	0.16	23
18.	Yellow-throated vireo (<i>Vireo flavifrons</i>)	6	0.35	2.45	0.13	12
19.	Sinaloa wren (<i>Thryothorus sinaloa</i>)	8	2.60	7.95	0.25	(R. N. Brown, unpublished)
20.	Black-crested titmouse (<i>Parus atricristatus</i>)	9	1.27	3.75	0.25	24
21.	Black-throated sparrow (<i>Amphispiza bilineata</i>)	9	1.02	0.98	0.51	25
22.	Black-whiskered vireo (<i>Vireo altiloquus</i>)	9	0.51	1.57	0.25	12
23.	Cardinal (<i>Cardinalis cardinalis</i>)	9	1.80	6.90	0.21	26
24.	Gray vireo (<i>Vireo vicinior</i>)	12	0.21	1.39	0.13	12
25.	Hermit thrush (<i>Hylocichla guttata</i>)	13	1.59	6.98	0.19	19, 20
26.	Solitary vireo (Eastern) (<i>Vireo solitarius</i>)	13	0.35	2.09	0.14	12
27.	Song sparrow (<i>Melospiza melodia</i>)	15+	2.62	9.18	0.22	11, 27
28.	Solitary vireo (Western) (<i>Vireo solitarius</i>)	17	0.33	2.51	0.11	12
29.	Wood thrush (<i>Hylocichla mustelina</i>)	18	1.56	3.04	0.34	19, 20
30.	Yellow-green vireo (<i>Vireo flavoviridis</i>)	21	0.17	1.29	0.12	12
31.	Philadelphia vireo (<i>Vireo philadelphicus</i>)	25	0.40	2.47	0.14	12
32.	Willow warbler (<i>Phylloscopus trochilus</i>)	31	3.31	7.27	0.31	28
33.	Carolina wren (<i>Thryothorus ludovicianus</i>)	36	1.66	3.59	0.32	29
34.	Red-eyed vireo (<i>Vireo olivaceus</i>)	43	0.35	1.42	0.20	12
35.	American robin (<i>Turdus migratorius</i>)	L	1.93	2.28	0.46	(C. W. D. and R. E. L. unpublished)
36.	Catbird (<i>Dumetella carolinensis</i>)	L	0.27	0.19	0.59	30
37.	European robin (<i>Erithacus rubecula</i>)	L	2.18	3.27	0.40	31
38.	Mockingbird (<i>Mimus polyglottos</i>)	L	3.06	1.10	0.74	5
39.	Rose-breasted grosbeak (<i>Pheucticus ludovicianus</i>)	L	3.70	10.50	0.26	6

Solitary vireo is listed twice as the Eastern and Western populations have different song data

our list does not disqualify birds showing only "eventual" versatility—birds such as the cardinal which, though having a large repertoire, may sing only one or two songs for a period of time before changing to others. Some variability (for example, song length, number of repeated elements) is found even in the single song type of the chipping sparrow⁷, and Hartshorne, discussing Hutton's vireo as a possible exception to his hypothesis suggests that a slight "change of key" may render the single, repetitive song less monotonous over time. Nevertheless ranking versatility by measuring repertoire size seems a reasonable approach, particularly given the ability of spectrographic analysis to detect differences between song patterns.

The use of average values for song length and intersong interval may also conflict with Hartshorne, who cites instances of unusually continuous singing in versatile birds that normally sing quite discontinuously. There is no reason to suppose, however, that non-versatile discontinuous singers might not show similar examples of more rapid singing. If the monotony threshold hypothesis is to have practical predictive value, it should apply over the broad range of bird songs making up our averaged figures.

In spite of the lack of significance in the two comparisons, two significant correlations in the data were found. Song length and intersong interval gave a strong positive correlation ($r_s = 0.60$, $P < 0.05$), as shown in Fig. 2. This suggests that each species in fact tends to sing in a manner that uses a sound-silence ratio similar to other species, but using a different rhythm, or cadence. These differences in rhythm may be an important quality differentiating one bird's song from another. The same sort of relationship of sound to silence can be found in syllable structure of the cardinal—short syllables being followed by short pauses and longer syllables being followed by longer pauses⁸. Both sorts of data emphasise the fundamental rhythmical quality of song, and it is interesting to speculate that this quality may in part be related to the physiology of vocalisation in birds. The process of respiration perhaps places constraints on song and syllable lengths as well as the intervals between them⁹. Alternatively, these temporal rhythmical relationships may be related to the effectiveness of song in overcoming environmental noise. Repetitive songs, or elements of songs produced at predictable intervals, may provide a high degree of redundancy which would provide for more effective reception by listeners.

Finally, a negative correlation between interval length and repertoire size ($r_s = -0.39$; $P < 0.05$, Fig. 1) may be viewed as supporting Hartshorne's basic notion of temporal differences between versatile and non-versatile species, while suggesting that percentage of performance time is not a good measure of it. Longer intervals between songs in more versatile birds might easily lead a field observer to suppose that continuity, rather than cadence, correlates with versatility. In view of our findings, a relationship between versatility and percentage of performance time is by no means general or predictable.

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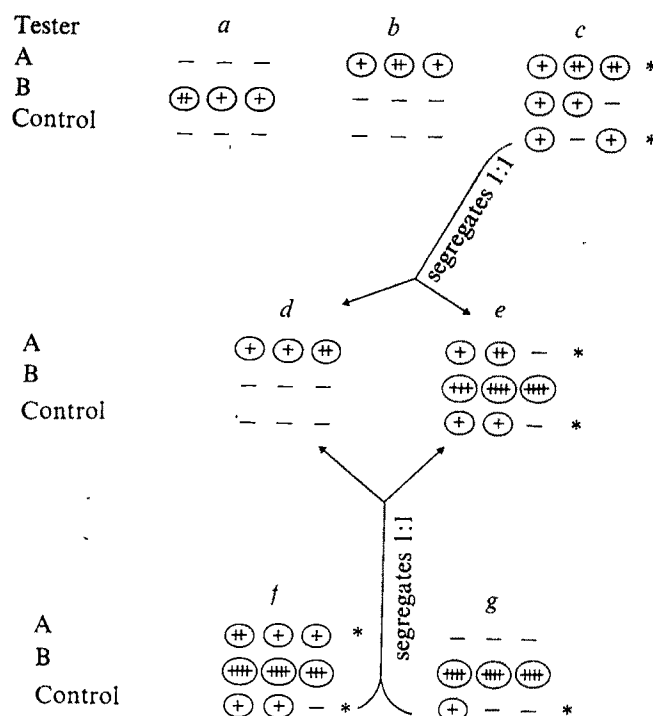
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Highly fertile form of the aggressive strain of *Ceratocystis ulmi*

THE ability to recognise the aggressive and non-aggressive strains of *Ceratocystis ulmi* in culture¹ has enabled us to survey the *C. ulmi* population in the field and thereby monitor any variation that might indicate a change in the course of the present epidemic of Dutch elm disease in Britain. When sampling from diseased elm twigs in the outbreak areas, we have found that while most wild isolates can be assigned to the two strains, there also exist in low frequency (about 2%) isolates with some affinity to the 'fluffy' aggressive strain but which produce small black-brown sclerotium-like bodies throughout the culture and are dark centred because of the presence of brown pigment. We show here that these isolates are a highly fertile form of the aggressive strain, with a special function in the perithecial, or sexual stage of the fungus.

Production of perithecia in *C. ulmi* usually requires the pairing of the two mating or compatibility types, A and B (ref. 2). During routine screening of wild isolates of the aggressive strain for compatibility type, after the method of Holmes³, autoclaved

Fig. 1 Mating reactions of *C. ulmi* isolated on elm twigs. Each diagram (a–g) represents the reaction pattern of an unknown isolate when tested against a standard non-aggressive A type, an aggressive B type, and on its own as a control, with three replicate twigs in each case. Perithecia after 1 month at 18 °C: —, nil; +, rare; ++, occasional; +++, frequent; +++, abundant. Reaction patterns: a, normal A type reaction (as found with non-aggressive isolates); b, normal B type (as found with non-aggressive and aggressive isolates); c, aggressive B type with pseudoselfing at *; d, F₁ fluffy B type, e, F₁ proto A type, with pseudoselfing at *; f, g, wild proto A types, with pseudoselfing at *.



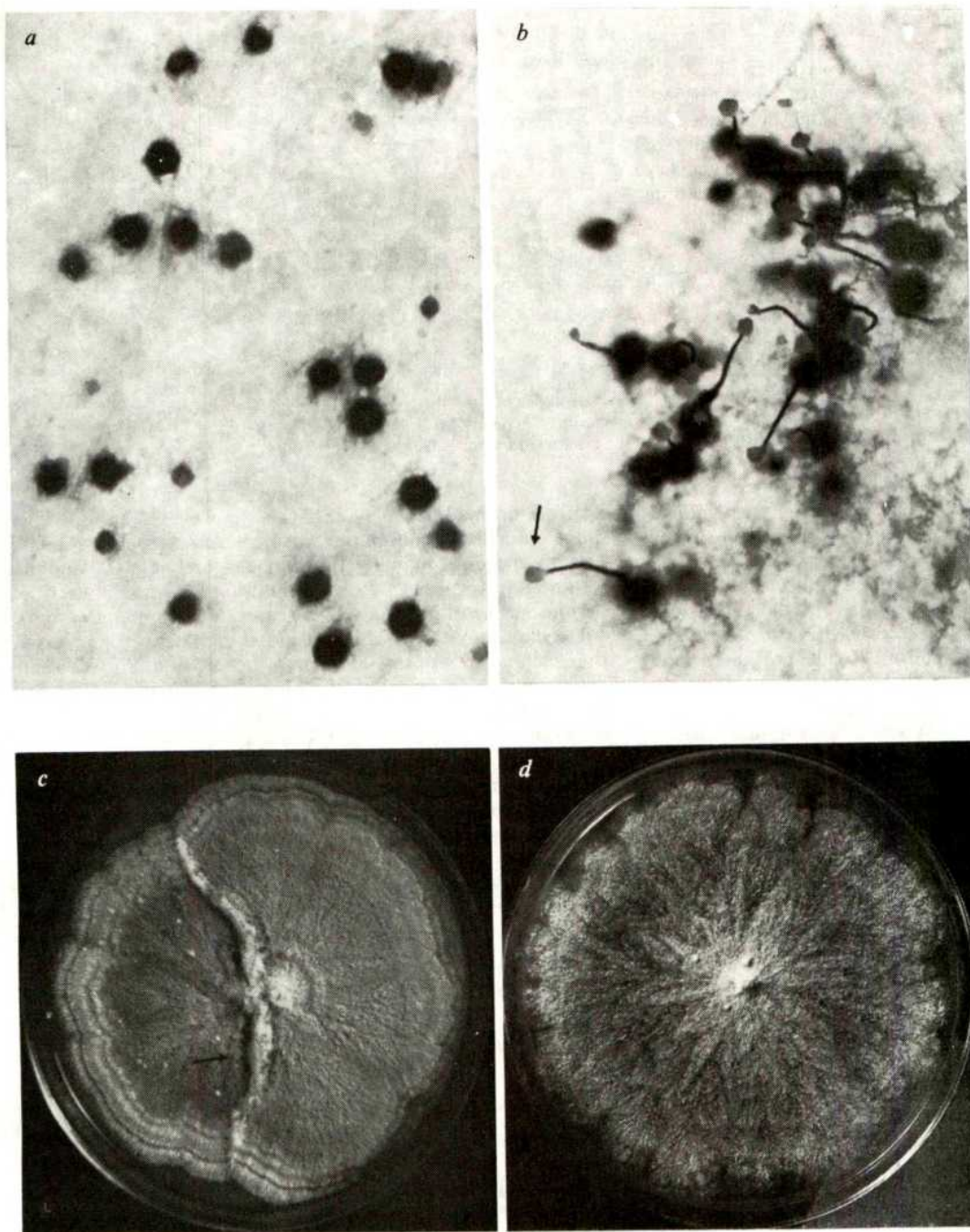


Fig. 2 Perithecium formation by *C. ulmi* on malt agar. *a*, Protoperithecia formed in a culture of a proto isolate (\times about 65). *b*, Mature perithecia formed at the junction of a proto and a fluffy isolate, showing typical long perithecial necks and oozing blobs of ascospores (arrowed; \times about 25). *c*, Formation of a black line of perithecia (arrowed) and a white felty zone line at the junction of a dark pigmented proto (left) and a fluffy (right) isolate. Culture incubated for 12 d in darkness at 18 °C, followed by daylight. *d*, Wild proto isolate S6. Culture incubated in darkness at 18 °C.

peeled elm twigs were dipped first in shake cultures of the unknown isolate, and then after incubation for 1 week at 18 °C, dipped again in a shake culture of a standard A or B tester strain. Control twigs were dipped in the unknown isolate only. No isolate gave a type A reaction (Fig. 1*a*) and most gave a clear-cut type B reaction, forming perithecia with the A tester only (Fig. 1*b*). Some isolates, however, while behaving as type B, occasionally formed scattered perithecia on the control twigs (Fig. 1*c*). As all isolates tested were originally derived from single spores, this 'selfing' phenomenon was particularly curious. Ascospores from such 'selfed' perithecia of three isolates, G11, G29 and S46 were therefore spread on malt agar, and retained on the bench top for observation. After 2 weeks the resulting colonies were seen to be producing abundant fertile perithecia (Fig. 2*b*), an interesting development as perithecia have normally been obtained only on sterilised elm bark or sapwood. The perithecia seemed to be forming at the junction of light and dark coloured colonies as a result of the maturation of numerous small spherical black-brown 'protoperithecia' (about 30–55 μ m, Fig. 2*a*).

To investigate the phenomenon in more detail, fifty single ascospore germings picked off from further spreads of isolate G11 were examined for growth rate and cultural characters on 2% Oxoid Malt Extract Agar at 18 °C. They varied significantly in growth rate, with a range which fell within that normally encountered in aggressive isolates (Fig. 3*a*). On the basis of culture morphology, however, they fell into two groups in a nearly 1:1 ratio. Isolates in one group resembled the parent aggressive isolate in their fluffy morphology¹. Isolates in the other group had some fluffy characteristics but, in contrast, were dark pigmented and also formed considerable numbers of protoperithecia. These isolates were therefore called protoperithecial ('proto'). The mean growth rate of the proto progeny (4.05) was significantly lower ($P < 0.01$) than that of the fluffy progeny (4.25). Ascospore progeny of two other isolates, S46 and G29, also fell into the two groups. With G29 progeny behaviour was similar to that for G11 but in S46 complete separation of the proto and fluffy progeny occurred for growth rate, with the proto isolates growing very much more slowly (Fig. 3*b*). When F_2 ascospores of G11 and S46 were sampled

from perithecia developing in the F_1 ascospore spreads, the process repeated itself, the progeny again separating into proto and fluffy groups.

As Fig. 3 shows, there was considerable variation in growth rate within the two groups. Variation also occurred in morphology, for example, in the pigmentation and abundance of protoperithecia in the proto form, and in the appearance of the aerial mycelium in the fluffy. This variation was particularly marked in the F_1 line of G11 derived by mixing and spreading the ascospores of 20 perithecia from first the F_2 and then the F_3 lines. Several new cultural types occurred within both groups.

The presence of perithecia at the junction of light and dark colonies suggested the involvement of both proto and fluffy progeny types in perithecial formation. A fluffy progeny isolate was therefore paired with its proto sibling on malt agar. Where the two colonies met, a zone of dense white felty mycelium was formed, and later a line of fertile perithecia developed (Fig. 2c). The ascospores again segregated 1:1 for the two types. Whatever their origin, any combination of proto \times fluffy progeny isolates formed perithecia, but fluffy \times fluffy and proto \times proto pairings did not. Furthermore, any wild fluffy isolate behaved in the same way as the fluffy progeny isolates. This suggested either stimulation of selfing, or cross fertilisation between the fluffy and proto types. When a proto progeny culture was seeded with conidia of a fluffy isolate, perithecia sometimes developed at the site of conidial application. This did not happen when a fluffy progeny isolate was seeded with a proto isolate, indicating that in a proto \times fluffy pairing, it is the protoperithecia of the proto culture which mature. Ability of the fluffy to fertilise the proto was demonstrated when ascospores resulting from the seeding of protoperithecia with a fluffy isolate tolerant to the fungicide MBC (ref. 4) segregated for MBC tolerance.

These results suggested a difference in compatibility type between fluffy and proto isolates. In tests of F_1 progeny of G11 and S46 on elm twigs the fluffy isolates gave a normal B-type reaction (Fig. 1d). Proto isolates gave a very strong A-type reaction and also formed numerous perithecia in patches on the control and A tester twigs (Fig. 1e). This showed that the proto is a highly fertile form of the A compatibility type and has, in addition, a marked tendency to 'self'.

It was already evident that proto progeny obtained from 'selfed' aggressive isolates closely resembled in morphology the dark sclerotial types found occasionally in nature (Fig. 2d). This similarity was confirmed when: (1) pairings between dark sclerotial (hereafter called 'wild proto') isolates and fluffy isolates on malt agar resulted in the formation of fertile perithecia; (2) progeny from these perithecia segregated 1:1 for proto and fluffy; (3) wild proto isolates showed a strong A compatibility reaction with a tendency to 'self' on control twigs (Fig. 1f and g); and (4) ascospores from such 'selfed' perithecia segregated 1:1 for proto and fluffy. One such wild proto isolate (T7) with a slow growth rate (about 2.8 mm d⁻¹) produced typical fast-growing fluffy progeny (range of five isolates 3.1–3.8 mm d⁻¹) as well as slower growing proto progeny (range of five isolates 2.7–3.2 mm d⁻¹).

This indicates that the fluffy type can be derived from a proto parent as well as the proto type from a fluffy parent and highlights the need to understand the mechanism by which this occurs. The evidence cannot be reconciled with the non-parental type having arisen by true selfing in a diploid or polysomic line, a situation which would in any case be most unusual in an ascomycete. Indeed the consistent 1:1 segregation of the two types suggests instead a single gene difference in a haploid. In this situation, the non-parental genotype could either arise by mutation or be pre-existing within the parent as a component of a heterokaryon. Because the isolates examined were derived from single uninucleate conidia⁵, and because successively 'singled' proto isolates 'self' on twigs, the origin of the phenomenon in heterokaryosis seems unlikely. On present evidence we prefer the hypothesis that within the fluffy B type mutation to proto A type occurs at a single locus. Proto nuclei arising within the mycelium of the vegetatively growing fluffy isolate on malt

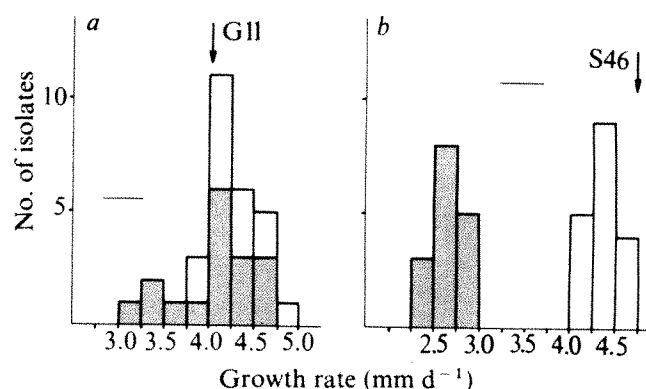


Fig. 3 Growth rate distribution of F_1 progeny derived from 'selfed' perithecia of aggressive isolates of *C. ulmi*. a, Progeny of isolate G11, b, Progeny of isolate S46. Proto progeny stippled, fluffy progeny white. Arrows show parental growth rates, and bars indicate least significant difference between isolates at $P < 0.05$.

agar are normally suppressed. When, however, an elm twig is dipped in a shake culture (in which a yeast-like budding phase occurs) spores containing proto nuclei are able to develop, resulting in localised A \times B fertilisation and maturation of perithecia. Resulting ascospores segregate 1:1 mutant (proto): wild type (fluffy). With this 'pseudoselfing', background variation such as growth rate among both proto and fluffy progeny could be due to segregation of mutations at other loci, and perhaps to some cytoplasmic variation. A similar explanation based on the reverse mutation could apply to the origin of the fluffy from the proto type.

Preliminary data suggest that the postulated proto locus has some influence on pathogenicity as well as on growth rate and culture morphology. An inoculation experiment on 5-yr-old clonal English elm, *Ulmus procera*, using techniques described earlier¹ with four replicates, showed that while three wild proto isolates caused very much more defoliation than two non-aggressive isolates, they caused significantly less ($P < 0.05$) than twelve wild fluffy aggressive isolates; mean percentage defoliation at 8 weeks being 2.3% for the non-aggressive, 50.8% for the proto and 75.9% for the fluffy isolates.

The occurrence of the protoperithecial form of the aggressive strain, and the phenomenon of pseudoselfing, reveal previously unknown properties in *C. ulmi* with considerable ecological implications. Perithecia occur naturally in beetle breeding galleries within the bark of diseased elms and the ascospores, together with asexual spores, are thought to be carried by the newly emerging beetles in the spring. Until now it has been assumed that for perithecial formation the absence of an aggressive A type in Britain would mean that the aggressive strain would have to hybridise with the non-aggressive strain, leading to a decline in pathogenicity⁶. It is now clear that this is not the case and that an aggressive B type could undergo pseudoselfing to produce two progeny genotypes, one the fluffy B form, close to the parent itself, and the other the highly fertile proto A form. Furthermore, in elm bark the latter would presumably produce abundant protoperithecia, which would be available for fertilisation, whether by pseudoselfing or by outcrossing with available B types. It seems that the aggressive strain could thus maintain itself even where perithecial formation rather than asexual reproduction was at a premium. The natural balance between the two forms would presumably depend on the relative selection pressures towards such attributes as perithecial formation, saprophytic ability within bark and pathogenicity.

The role of the proto form in the present epidemic is being investigated. The low frequency of proto isolates in twig samples suggests that in the pathogenic phase of the fungus this form is presently less fit than the fluffy form. Some perithecia from bark in the main outbreak areas have, however, produced equal numbers of fluffy and proto progeny. Furthermore, a reinvesti-

gation of the ascospore progeny from the perithecia found in beetle galleries of the imported rock elm logs examined in January 1973 (ref. 7) has shown that these too consist of proto and fluffy forms. Thus these perithecia must have resulted from proto \times fluffy outcrossing or pseudoselfing.

Our laboratory experiments have shown that pseudoselfing and proto \times fluffy outcrossing can result in significant variation among progeny. In nature this could lead to the appearance of new genotypes, and in a situation in which selection for aggressiveness were relaxed, might well contribute to a decline in the pathogenicity of the present *C. ulmi* population and thus of the epidemic.

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Morphine withdrawal response and central cholinergic activity

MOST of the different neurotransmitter systems have been implicated in opiate withdrawal. Frederickson and Pinsky¹ suggested a primary role for acetylcholine (ACh) in both the development and the expression of dependence on morphine but the specific role of cholinergic mechanisms in opiate withdrawal is not yet clear and deserves further attention. The hypothesis of the role of ACh in the expression of withdrawal phenomena, on which most earlier experiments were based, was that of the 'cholinergic excess', first postulated by Paton². Tests of this hypothesis in various laboratories have, however, produced conflicting results and thus confusion rather than clarification of the role of cholinergic mechanisms in the withdrawal syndrome³⁻⁹. Unfortunately, many such studies have neglected to differentiate between peripheral interference with expression and central interference with initiation of the signs of withdrawal by the various drugs studied. Our experiments were designed to eliminate this particular factor, which has contributed to much of the conflict. The results clearly illustrate the confounding effect of failure to separate peripheral from central cholinergic effects and suggest that the exact opposite of the cholinergic excess theory is the actual nature of the central cholinergic role in the morphine withdrawal syndrome.

Male albino rats (Sprague-Dawley, Harlan, 140-160 g) were made morphine-dependent by the administration of a slowly absorbed suspension of morphine base in mannide monooleate (300 mg kg⁻¹, subcutaneously) as described previously^{8,10}. Withdrawal was precipitated 48 h later, which is the approximate time of peak withdrawal response¹⁰, by the injection of naloxone (0.03-3.0 mg kg⁻¹, subcutaneously) and withdrawal severity was assessed by a point-scoring technique modified slightly from that described previously¹⁰ by weighting the signs. The range of scores possible for each sign is given in the legend to Fig. 1. In the present experiments the withdrawal signs scored were grouped before analysis as follows. Group 1: those

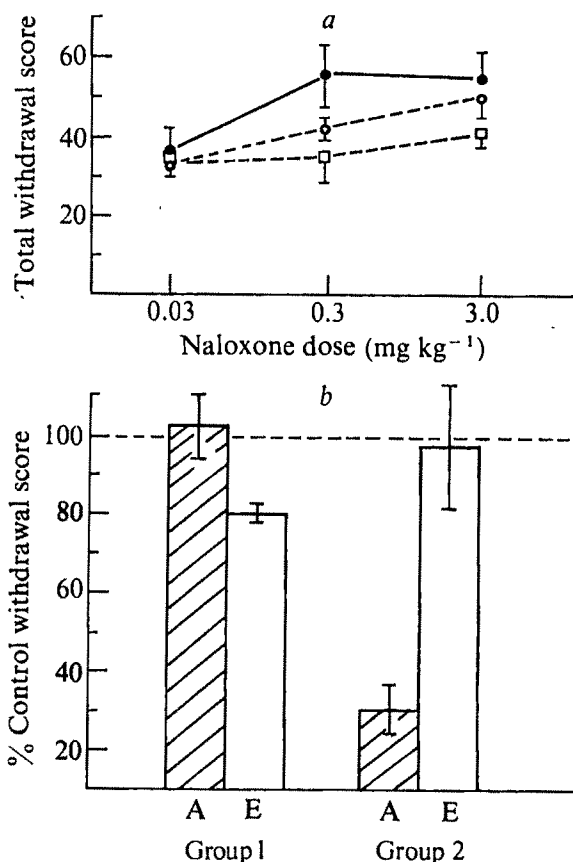


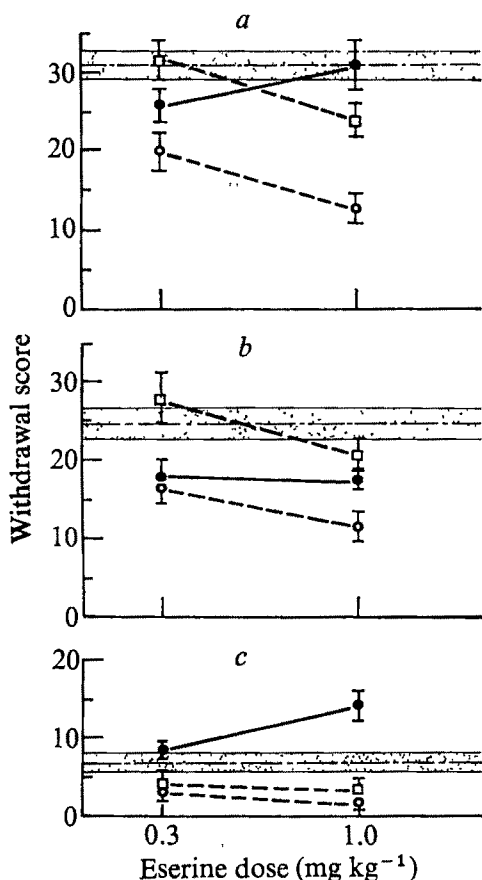
Fig. 1 *a*, The effects of eserine sulphate (0.25 mg kg⁻¹, intraperitoneally, (□) and atropine sulphate (5 mg kg⁻¹, intraperitoneally, (●)) on the severity of the naloxone-induced withdrawal response of morphine-dependent rats. The points refer to the means (± s.e.) of the total withdrawal score per animal ($n = 6$ rats per point). The signs scored, with the range of possible scores for each sign, follow; group 1 signs: wet dog shakes (0-12), escape jumping (0-36), escape digging (0-12), yawning (0-12), ptosis (0-8), penile erection (0-12), irritability to handling (0-6), reaction to poking with sharp object (0-12), restless activity (0-8); group 2 signs: rhinorrhea (0-8), lacrimation (0-12), salivation (0-18), diarrhoea (0-8), seminal emission (0-8). Control animals received saline before naloxone. *b*, Effects of eserine sulphate (0.25 mg kg⁻¹, open bars) and atropine sulphate (5 mg kg⁻¹, hatched bars) on group 1 as compared with group 2 signs. The data are presented as percentages (mean ± s.e.) of 100%. The scores for the group 1 signs and the scores for the group 2 signs were determined separately. The data presented in this figure were obtained from a total of 54 rats, 18 per treatment mode. A, Atropine sulphate; E, eserine sulphate.

signs with a strong voluntary component which were found to be influenced by muscarinic or antimuscarinic drugs by action in the central nervous system; and group 2: those signs with a strong autonomic or peripheral parasympathetic component whose peripheral expression was found to be influenced by muscarinic or antimuscarinic drugs by action in the periphery. The actual signs used for each analysis are given in the legend to each figure. Eserine sulphate and atropine sulphate were injected intraperitoneally 30 min before the challenge with naloxone. When combined treatment was used, either atropine sulphate or atropine methylnitrate was injected intraperitoneally immediately before eserine sulphate.

The total withdrawal score precipitated by various doses of naloxone (0.03-3.0 mg kg⁻¹, subcutaneously) was slightly diminished by treatment with eserine at 0.25 mg kg⁻¹ and similarly with atropine at 5 mg kg⁻¹ (Fig. 1*a*). The apparent paradox of a cholinergic agent, eserine, and an anticholinergic agent, atropine, producing similar effects can be explained if the different possible sites of action are considered. Thus, when the withdrawal signs were separated as described above, it was

apparent atropine did not reduce the severity of the group 1 signs but markedly reduced the score for group 2 signs, many of which are parasympathetic in nature (Fig. 1b). Thus atropine seems to reduce withdrawal severity merely by blocking the peripheral expression of the subset of signs with a strong peripheral parasympathetic component. Conversely, eserine, at the dose used, had no effect on the peripheral signs but did reduce the severity of central signs (Fig. 1b). These results predict that the combination of a central cholinergic agent with peripheral cholinergic blockade should reduce the withdrawal response very effectively. Thus, in the presence of methylatropine, eserine should effectively alleviate overall withdrawal severity. Methylatropine blocks muscarinic receptors in the periphery but does not readily penetrate the blood-brain barrier. We did, in fact, observe a marked dose-dependent reduction in withdrawal severity of morphine-dependent rats after combined treatment with eserine and methylatropine (Fig. 2a). Eserine alone did not have such an effect. The importance of the central cholinergic activity of eserine in reducing the withdrawal response became evident when the effects of combined treatment with eserine and atropine sulphate were examined. Atropine sulphate should have the same actions peripherally as methylatropine, yet, the combination of eserine and atropine did not have the same ameliorating effect on withdrawal as did the combination with methylatropine (Fig. 2a). This is interpreted as attributable to antagonism of the central cholinergic actions of eserine by atropine. An examination of group 1 and group 2 signs separately further confirmed this interpretation (Fig. 2b, c).

Fig. 2 The effects of eserine sulphate (0.3 and 1.0 mg kg⁻¹ subcutaneously) alone ● and in combination with atropine methyl-nitrate ○ or atropine sulphate □ on (a), total withdrawal score, (b), group 1 withdrawal score; and (c), group 2 withdrawal score. The stippled regions on each graph represent the control withdrawal score (mean ± s.e.). The signs used for the total, group 1 and group 2 scores were identical to those listed in the legend to Fig. 1. The data presented in this figure were obtained using 42 rats, 6 rats per point. Naloxone was injected at 0.3 mg kg⁻¹ subcutaneously to precipitate withdrawal. The dose of atropine methyl-nitrate and atropine sulphate used in these experiments was 5 mg kg⁻¹ intraperitoneally.



The higher dose of eserine increased the severity of the group 2 signs and both atropine and methylatropine blocked this effect (Fig. 2c). Thus eserine seems to increase the severity of group 2 signs by a muscarinic action in the periphery. Both doses of eserine reduced the severity of the group 1 signs (Fig. 2b). Atropine blocked this effect; methylatropine did not, but in fact increased the beneficial effect of the higher dose of eserine. These interactions clearly illustrate that eserine must alleviate the severity of group 1 signs by a muscarinic action in the central nervous system.

These results provide further evidence for an important role for acetylcholine in morphine withdrawal but do not support the hypothesis^{2,11,12} of a cholinergic crisis resulting from excessive release of ACh on to supersensitive receptors. On the contrary, the specific derangement in central cholinergic function which occurs after the administration of naloxone to a morphine-dependent animal seems to be a cholinergic deficit. Thus when the interference from cholinergic actions in the periphery is eliminated by methylatropine, eserine relieves the withdrawal response, presumably because of an increase in central muscarinic activity, as it is blocked by atropine.

Frederickson and Pinsky¹ reported that the syndrome which occurs on abstinence morphine withdrawal is biphasic in nature. The early phase seems to coincide with a cholinergic deficit since it is potentiated by anticholinergic treatment, while the later phase seems to have a cholinergic component, since its severity is reduced by treatment with anticholinergic drugs. Labrecque and Domino¹³ have reported a biphasic effect of naloxone also on cortical ACh release in morphine-dependent cats. ACh output was decreased at 15 min after 1.0 mg kg⁻¹ of naloxone while an increase was found at 45–105 min. Thus the naloxone-precipitated withdrawal response seems to be an accelerated and accentuated correlate of the spontaneous withdrawal syndrome. The studies of ACh release should be repeated on rats, but it is interesting that the withdrawal response precipitated by naloxone, reported here as being a result of a central cholinergic deficit, occurred dramatically within the first 15 min of naloxone injection, corresponding to the time the decrease in output of cortical ACh was observed by Labrecque and Domino¹³.

In conclusion, the present results provide an explanation for the previous conflicting reports³⁻⁹ of the effects of cholinergic and anticholinergic drugs on opiate withdrawal, since either an increase or a decrease in withdrawal severity could be expected, with either a cholinergic or an anticholinergic agent, depending on the sign or signs examined. The early phase of the opiate withdrawal response seems to correspond to a central cholinergic deficit and I suggest the most effective alleviation of withdrawal stress requires the unique combination of a central muscarinic action plus a peripheral muscarinic blockade.

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Low levels of photoreactivating enzyme in xeroderma pigmentosum variants

XERODERMA pigmentosum (XP) is a recessive, hereditary disease in which patients develop malignancies on areas of

Table 1 PRE and pyruvate kinase levels in normal and XP variant cells

Cell line	Pyruvate kinase* activity (% normal)	PRE activity† (% normal)
Normal		
HESM	100	100
Variants		
XP4BE	81.4	11
XP13BE	40.6	9
XP7TA	104	4
XP30RO	80.2	56

*Determined by the method of Ibsen and Trippet¹¹.†Cells were grown collected and assayed as described previously^{5,9}. Protein concentrations were determined by the method of Lowry¹². Typical levels of enzyme activity for normal cells were 600–620 pmol mg⁻¹ min⁻¹. All values were the average of at least three independent determinations except for XP30RO, which had two.

skin exposed to sunlight¹. Although fibroblasts from most XP individuals are deficient in excision repair of damage to DNA (classical XP), those from a few individuals (XP variants) having the usual clinical signs of XP seem to have normal excision capacity². Photoreactivation is a DNA repair process in which the photoreactivating enzyme (PRE) monomerises pyrimidine dimers induced by ultraviolet light³. As PRE is present in normal human cells⁴, but only at reduced levels in classical XP cells⁵, we thought that the XP variants may also be defective in PRE activity. We therefore measured this activity in fibroblasts from four XP variants (Table 1) and found that Variants XP30RO, XP4BE, XP13BE, and XP7TA have 56, 11, 9, and 4% of the normal level of PRE activity, respectively.

Are the lower levels of PRE an intrinsic property of the cells or an artefact of culture or assay conditions? The high levels of PRE activity in the normal cells have been shown to be neither an artefact of mycoplasma infection of the normal lines nor of age of the cell donor at biopsy⁵. We have also examined the possibility that the low PRE activity in XP variants may result from: systematic loss of enzyme activity with culture age; unfavourable enzyme assay conditions; presence of inhibitors of PRE activity; or slower growth rates of the XP cells leading to general enzyme depression. We found, however, no systematic decrease in PRE activity throughout 10 cell passages; neither an increase nor decrease in pH or ionic strength, nor the omission of Mg²⁺ from the assay mixture, increased the apparent enzyme activity in extracts of XP7TA; mixing experiments indicated that the cell extracts of XP7TA had no effect on the activity of the normal human enzyme; and pyruvate kinase (chosen as the representative enzyme of cellular metabolism other than DNA repair) levels were 40.6, 81.4, 104 and 80.2% of the normal level, for XP13BE, XP4BE, XP7TA and XP30RO, respectively. We conclude that these factors do not play a major part in determining the level of PRE activity measured in our assay. These data imply that the observed low levels of activity were not

artefacts of culture or assay conditions, and may contribute to the depression of total DNA repair capacity in XP variants.

What other processes must be considered in determining the total cellular repair capacity? In addition to photoreactivation, cells have two other pathways for repair of ultraviolet-induced damage to DNA: excision repair⁶ and postreplication repair⁷. Excision repair and photoreactivation act principally as prereplication error-correcting pathways, whereas postreplication repair works to seal gaps presumably generated opposite damaged bases during DNA replication. One measure of cellular excision repair ability is unscheduled DNA synthesis, the ultraviolet-induced incorporation of ³H-thymidine into nuclei of non-S-phase cells. XP4BE, XP13BE, XP7TA and XP30RO can carry out excision repair at almost normal levels (Table 2). Although quantitation of postreplication repair is difficult, Lehman *et al.*⁸ have shown that 1 h after ultraviolet irradiation, the DNA of XP variants is much smaller (and thus contains more unrepaired gaps) than that from normal cells. The data of Lehman *et al.*⁸ for the relative sizes of the DNAs at 1 h postirradiation are shown in Table 2. PRE activity (Table 2) was measured as described previously^{5,9}.

We obtain a rough estimate of the total repair capacity of a cell by adding the values for each of the three processes. The two clinically normal individuals thus have total repair capacities of 300 (for HESM) and 240 (for CeAr). The latter individual is the mother of one normal and five XP children; although she (like other presumed heterozygotes for excision repair¹) has normal unscheduled synthesis, her PRE level is about half normal. This finding indicates that a small loss of repair capacity may be tolerated without apparent detriment.

XP30RO has only mild to moderate clinical symptoms of XP and an intermediate repair capacity of 173, whereas variants with severe symptoms (XP4BE, XP13BE and XP7TA) have even lower repair capacities—143, 141 and 137, respectively.

It is likely that the three repair processes are not equally important in cellular DNA repair *in vivo*. Assignment of a 10-fold greater importance, however, to excision repair than to postreplication repair and photoreactivation led to predictions of similar repair capacities for normal and XP variants, in contradiction to their clinical symptoms. Similarly, assignment of 10-fold greater importance to photoreactivation or postreplication repair led to the prediction that CeAr should be XP instead of normal, or that all XP variants should show the same degree of affliction with XP respectively. It is thus possible that all three repair processes are necessary for normal repair—perhaps because of the lack of overlap of the repair processes or availability of the DNA to the repair enzymes in space or time¹⁰.

In spite of the apparent correlation of the clinical symptoms with the calculated total repair capacity, at least three variables—differential sun exposures of individuals to sun, variable shielding by pigments in the damage and differing ages on clinical examination—make the use of such a simple scheme of computation of DNA repair capacity difficult. The apparent

Table 2 Repair capacities in normal and XP cells

Cell line	Phenotype	Unscheduled* DNA synthesis (% normal)	Relative† DNA size after irradiation (% normal)	PRE Activity (% normal)	Total repair capacity
Normal					
HESM	Normal	100§	100§	100	300
CeAr‡	Normal (ref. 1)	100 (ref. 1)	100§	34	234
Variants					
XP30RO	Light/moderate XP (ref. 8)	88 (ref. 8)	29.2 (ref. 8)	56	173
XP4BE (Wo Mec)	Severe XP	100 (ref. 8)	32§	11	143
XP13BE (Pe Hay)	XP	100 (ref. 1)	32§	9	141
XP7TA	Severe XP	99 (ref. 8)	34 (ref. 8)	4	137

*Unscheduled DNA synthesis is a measure of excision repair¹.

†Relative DNA size 1 h after ultraviolet irradiation is a measure of postreplicative repair ability.

‡Parent of one normal and five XP children¹.

§Although these cell lines were not tested, the values shown are typical for normal (HESM and CeAr) and XP variant (XP13BE) cells, respectively.

correlation of DNA repair capacity with clinical signs or propensity to sunlight-induced skin malignancy—and the severity of such disease—makes it tempting to speculate that there is a direct relationship between the total DNA repair capacity and development of sunlight-induced skin cancer.

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Rat liver microsomes catalyze covalent binding of ¹⁴C-vinyl chloride to macromolecules

THE occurrence of angiosarcoma and injury of the liver in workers occupationally exposed to vinyl chloride (VC) has focused interest on the fate of VC in the organism. Jaeger *et al.*¹ presented evidence that pretreatment of rats with phenobarbital, which induces the microsomal mixed function oxidase system of the liver, also enhances liver toxicity of VC. They suggested that VC may be transformed by this enzymic system to a reactive epoxide intermediate which has also been considered by some other authors^{2,3}. This intermediate, according to its electrophilic properties, might interact with components of the liver cell. We present here some results which further support this theory.

We incubated 1,2-¹⁴C-VC with rat liver microsomes and an NADPH-regenerating system (isocitrate and isocitric dehydrogenase). Preparation of microsomes and the incubation mixture followed standard procedures⁶. The incubation vessels were connected to an all-glass system containing the radioactive VC substrate which was diluted with atmospheric air, so that incubations could be carried out at different rates of supply of the gaseous substrate. After incubation (90 min) the total uptake of substrate during incubation was determined by measuring the total radioactivity present in the incubation mixture. Irreversible binding of VC metabolites to the microsomal protein was determined after exhaustive solvent extrac-

tions as described previously using other lipophilic substrates^{4–8}. It is known that rat liver converts VC to metabolites such as chloroethanol, chloroacetaldehyde and chloroacetic acid^{3,7}. Therefore extraction of the proteins had to remove the lipophilic VC and the water-soluble metabolites. Exhaustive extraction of the microsomal proteins was achieved using a schedule originally developed for removing metabolites of imipramine⁸. This included precipitation of proteins with ethanol and washing of the precipitate with 70% ethanol, Tris buffer, pH 7.5 (twice), 70% ethanol, absolute ethanol (twice), acetone-chloroform (4:1, twice), and finally 70% ethanol. Irreversible binding of VC metabolites to soluble proteins and nucleic acids which had been added to the microsomal incubation was estimated after adsorption of the lipophilic VC to charcoal (norit A) and subsequent dialysis of the water-soluble metabolites. After these procedures no further radioactivity could be removed from the soluble proteins or nucleic acids if these were subjected to solvent extraction (70% ethanol at –20 °C). The radioactivity which was attached to the protein remained constant during this extraction procedure.

Table 1 shows that supply of NADPH was essential to achieve covalent binding of VC metabolites to rat liver microsomes. In addition, VC metabolites were also bound to albumin when this was added to the microsomal incubation. Non-mercapto-albumin was prepared according to Carlsson and Svenson⁹. This preparation was much less efficient in trapping the reactive intermediate formed from VC by the microsomal enzymes. Concanavalin A (con A), containing no cysteine at all, does not covalently bind VC metabolites, indicating that cysteine participates in the binding reaction. The results are in agreement with the assumption of VC-epoxide being the reactive VC metabolite. Similar conclusions have been drawn for metabolic activation of vinylidene chloride⁹.

Further experiments fitted this concept. Covalent binding of VC metabolites to proteins in our system was depressed by 30% on addition of 1 mM glutathione. Addition of cytosol, which contains glutathione, had a similar effect.

The inhibitor of microsomal cytochrome P450-dependent mixed function oxidations, 1-naphthyl-4(5)-imidazole¹⁰ at a concentration of 10^{–4} M, inhibited covalent binding by 85%.

The finding that VC metabolites can also be bound to RNA (Table 1) seems to be of particular importance with regard to the mechanism of chemical carcinogenesis induced by VC. It is commonly accepted that pre-carcinogens are primarily metabolised to electrophilic ultimate carcinogens which can bind covalently to cellular macromolecules¹¹.

Additional support for the involvement of epoxide in the covalent binding reaction could be obtained from experiments using the xanthine oxidase model system which generates H₂O₂ and the O₂[–] radical¹². These incubations (1 ml) contained: 10 mg albumin, 0.8 mM hypoxanthine, 8 mU xanthine oxidase (Boehringer, Mannheim) and 0.08 mM EDTA, and were exposed to the ¹⁴C-VC-containing atmosphere for 60 min. Control incubations with albumin and 5 mM H₂O₂ showed no covalent binding of ¹⁴C-VC to the albumin, whereas the complete xanthine oxidase system, when taking up 10 nmol ¹⁴C-VC,

Table 1 Covalent binding of metabolites of ¹⁴C-VC in rat liver microsomal incubations*

	Binding to microsomal protein (nmol VC metabolites bound to 1 mg microsomal protein)	Binding to proteins and RNA added to the microsomal incubations (nmol VC metabolites bound to 10 mg macromolecular compound, as catalysed by 1 mg microsomal protein)†			
		Bovine serum albumin‡	Non- mercaptoalbumin§	Con A¶	RNA
Without NADPH-regenerating system	<0.001	<0.001	<0.001	<0.001	<0.001
With NADPH-regenerating system	0.14 ± 0.07 (mean ± s.d.; n = 9)	0.15	0.07	<0.001	0.12

* 90 min, 37 °C. Volume of incubation = 1 ml. Uptake of substrate (VC) = 10 nmol in presence of NADPH

† Mean values of double determinations.

‡ Contains cys-SH and cys-SS-cys.

§ Contains cys-SS-cys.

¶ Contains no cys.

bound 12.6 ± 1.2 pmol (mean \pm s.d.; $n = 3$) VC metabolites to the 10 mg albumin. This strongly suggests that O_2^- radicals, which are known to be involved in epoxidation by the microsomal enzyme system¹³, convert VC to a metabolite which then binds covalently to a protein-like albumin.

In accordance with our results Bartsch *et al.*^{9,14} reported that in bacterial test systems VC and also the closely related compound vinylidene chloride are only found to be mutagenic if rat liver microsomes are present to form the ultimately mutagenic metabolite. Pretreatment of the rats with phenobarbital enhances the mutation rate. This also corresponds to the work of Jaeger *et al.*¹ on liver toxicity of VC in phenobarbital-pretreated rats. Thus, the concept of a reactive metabolite of VC involved in carcinogenesis and liver toxicity gains further support.

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A genetic basis for resistance to enteric disease caused by *E. coli*

THE K88 antigen on the surface strains of *E. coli* which cause neonatal diarrhoea in piglets^{1,2} is an essential virulence determinant^{3,4} because its adhesive properties enable K88-positive strains to attach to piglet intestinal mucosa^{4–7} and establish in the small intestine⁴. These bacteria multiply to reach abnormally large numbers in the gut⁸ and synthesise enterotoxins that cause diarrhoea and death of infected piglets^{9,10}. K88-positive bacteria, however, do not attach to intestinal tissue from all piglets, and at least two pig phenotypes occur^{6,7}, which we have designated 'adhesive' (signifying that K88-positive bacteria attach to their brush borders) or 'non-adhesive' (K88-positive bacteria do not attach to their brush borders). Phenotypes are the products of two alleles at a single locus which are inherited in a simple Mendelian manner⁷, and the phenotypes of progeny from selected matings support the conclusion that the product of the 'adhesive' allele is dominant over the product of the 'non-adhesive' allele (R. A. G., R. S. and J. M. R., unpublished).

Thus, it seems reasonable to postulate that K88-positive bacteria colonise the gut of 'adhesive' pigs more readily than the gut of 'non-adhesive' pigs, and that 'adhesive' animals are more likely to be susceptible to neonatal diarrhoea caused by these strains. To investigate this possibility, five litters comprising 47 piglets were removed from their dams 2 d after birth

and fed on cow's milk¹¹. Two days later, 1 ml of a suspension containing 10^{10} viable organisms of a virulent K88-positive strain W1 (0149: K91 (B), K88ac(L); H10) of *E. coli* was given to each piglet by stomach tube. Clinical observations were made daily and severely diseased piglets either died or were killed *in extremis* within 4 d of challenge; healthy surviving piglets were killed 5–7 d after challenge. All piglets were necropsied and viable bacterial counts were made on the contents of the intestine. Brush borders were prepared from the small intestine of each piglet and its phenotype was determined⁶.

Twenty-three piglets from the five litters were phenotyped as 'adhesive' and 21 of these piglets died or were killed with clinical signs of neonatal diarrhoea. At necropsy, strain W1 was present in large numbers throughout the gut and was generally dominant in the anterior small intestine. The two 'adhesive' piglets which survived showed no clinical signs of disease and did not excrete strain W1 after challenge.

Twenty-four piglets from the five litters were phenotyped as 'non-adhesive' and 23 survived challenge. These piglets showed no clinical signs of neonatal diarrhoea and did not excrete strain W1. One 'non-adhesive' piglet died 72 h after challenge with mild signs of neonatal diarrhoea but excreted only small numbers of strain W1.

The mean \log_{10} viable counts of *E. coli* in the anterior small intestine of the 23 healthy 'non-adhesive' survivors was 5.8; strain W1 (\log_{10} 5.3) was present in only one of these animals. In contrast, the mean count of strain W1 was \log_{10} 8.2 in 15 diseased 'adhesive' piglets that were killed, and \log_{10} 9.7 in six diseased 'adhesive' pigs that died. In the two healthy 'adhesive' pigs which survived, strain W1 was not detected in the intestine, and in the diseased 'non-adhesive' piglet which died, strain W1 was present in the anterior small intestine (\log_{10} 7.6) but was outnumbered almost 100-fold by other *E. coli*.

These results indicate that the 'adhesive/non-adhesive' phenotype determines whether the K88-positive enteropathogenic strain establishes in the gut and proliferates to cause clinical disease. In the 'adhesive' animals, strain W1 attaches and multiplies to reach large numbers in the small intestine, whereas in the 'non-adhesive' animals, strain W1 is unable to attach and rapidly disappears from the gut. The survival of two 'adhesive' piglets in which strain W1 failed to establish demonstrates, however, that additional factors may prevent attachment and proliferation of K88-positive bacteria in the gut. The death of one 'non-adhesive' piglet with strain W1 in the small intestine indicates that attachment by K88 is not always essential for the pathogenic strain to establish in the gut.

The complex nature of the *in vivo* situation has been emphasised in an experiment in which the expected susceptibility of sucking 'adhesive' piglets experimentally infected at birth was not expressed; these piglets received colostrum from the dam that contained K88 antibodies which are known to be associated with passive protection of piglets against challenge with strain W1^{12–14}. It seems reasonable to suggest that these 'adhesive' piglets were susceptible to infection but passive protection conferred by the K88 antibodies in colostrum and milk prevented the enteropathogenic strain from attaching and multiplying to reach high numbers in the gut.

These results have a number of interesting implications. It seems that the 'non-adhesive' phenotype primarily determines whether an animal will be resistant to infection with K88-positive strains of *E. coli*; however, if 'adhesive' animals receive protective antibodies in mammary secretions, then they may seem to be 'resistant' to infection. Although the nature of the product of the 'adhesive' allele is unknown, study of the K88 antigen has provided, as far as we are aware, the first example of a genetic basis for resistance to enteric disease. As a result, it may be possible to select breeding pigs that have the 'non-adhesive' gene and thus produce progeny resistant to neonatal diarrhoea caused by K88-positive *E. coli*.

In addition to this practical application, it is becoming clear that adhesion to tissue surfaces may be an important mechanism of pathogenicity in several diseases including human bacterial

infections caused by *Vibrio cholerae*¹⁵, and streptococci¹⁶. If the principles that we have demonstrated for K88-positive *E. coli* infection^{4,6,7,12-14} apply more generally, then the outcome of such infections may be determined by the virulence determinants of the microorganism, the phenotype of the host and the immune response of the host or its dam. An understanding of the genetic susceptibility of the host may, therefore, be an essential prerequisite for the interpretation and evaluation of infection experiments with those pathogens which become attached to cell surfaces.

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Cadmium resistance and content of cadmium-binding protein in cultured human cells

INDUCTION of hepatic metallothionein (a cadmium-binding protein) is considered to be a protective mechanism in mammals against the toxic cadmium ion. Pretreatment of rats with low doses of cadmium induces the synthesis of metallothionein and also protects against subsequent exposure to an otherwise lethal dose¹. But the role of metallothionein in cadmium resistance is not clear². Restriction of food intake in the rat increases the level of metallothionein³, but does not alter the LD₅₀ of cadmium². Also the synthesis of metallothionein seems to continue for a longer time than the protection against cadmium in both rats and mice^{1,2}. Lucis, Shaikh and Embil⁴ found uptake of Cd in cultured human embryonic fibroblasts, HeLa cells and monkey kidney epithelial cells after 8 d of incubation with Cd. At that time the cells also contained a Cd-binding protein. The authors did not, however, describe growth of the cells in the presence of Cd or development of cell strains resistant to Cd. Webb and Daniel⁵ recently described the synthesis of a Cd-binding protein in cultures of cells derived from the cortex of the adult pig kidney.

We describe here the development of Cd resistance and the relationship between cadmium resistance and cadmium-binding protein (CdBP) levels in cultured human skin epithelial cells.

Human skin epithelial cells (HE cells) NCTC strain 2544 were obtained from American Type Culture collection, Rockville, Maryland and grown in Falcon plastic tissue culture flasks (75 cm²) in Dulbecco's modified Eagle's medium supplemented with foetal calf serum and horse serum as described in ref. 6. Medium was changed every 3 d. Cadmium (20 µM) added to the medium had toxic effects; after 2-3 d the cells altered shape, about 90% of them died and the growth of those remaining ceased. After a lag period of about a week, the cells retained their growth. The Cd concentration was kept at 20 µM and after 5-6 weeks the cells had regained a normal appearance and an almost normal growth rate, that is, a generation time of about 24 h. At that time the Cd concentration was increased

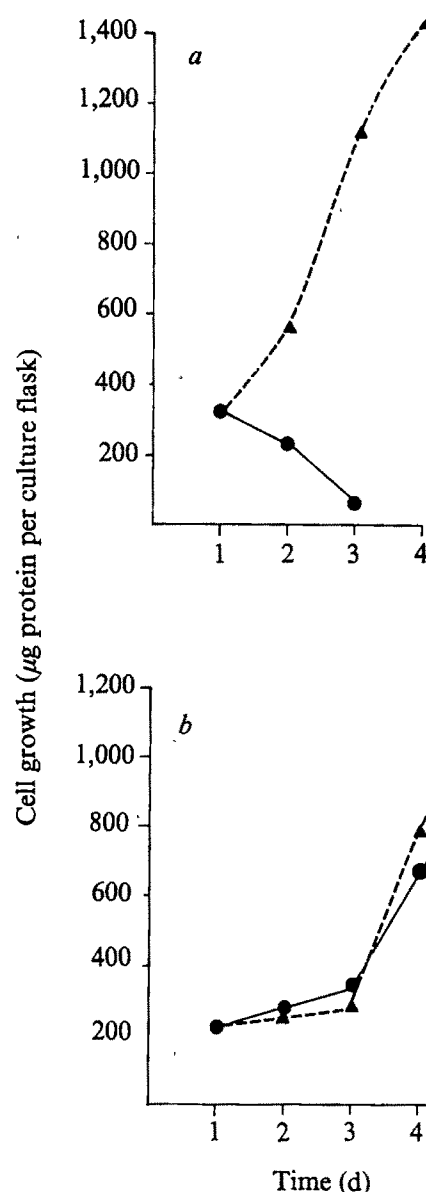


Fig. 1 Growth of human epithelial cells not made resistant to cadmium (a) and human epithelial cells made resistant to 100 µM Cd in the growth medium (b). Both cell lines were grown in medium without Cd added (▲) and with 100 µM Cd added (●).

to 40 µM. The cells again altered morphology, most of them died and the growth ceased temporarily, but in the course of some weeks, the cells regained normal growth. By increasing the Cd concentration in the medium about every 6 weeks, the cells were made resistant to 100 µM Cd in about 30 weeks. After 30 weeks the resistant cells had about the same growth rate in the presence of 100 µM Cd as in Cd-free medium (Fig. 1). This concentration of Cd would kill HE cells not previously exposed to Cd. The resistant cells could be frozen in liquid nitrogen for several months without losing the resistance towards Cd. The cells could also be grown for at least 4 weeks without Cd in the medium without losing Cd resistance. These Cd-resistant HE cells have a generation time of approximately 24 h (like the parent cell line) and the resistance is thus persistent for at least 20 generations without any Cd added to the growth medium.

Growth medium and cells were tested for low molecular weight CdBP by addition of ¹⁰⁹Cd, a gamma emitter, to the cell cultures. To get a maximum uptake of labelled Cd, cultures of resistant cells were grown for at least 3 d in a Cd-free medium before the addition of ¹⁰⁹Cd. Labelled Cd (final concentration 1.3 µM) was then added to the growth medium. After growth

for 24 h in medium containing ^{109}Cd , the medium was collected, the cells were washed twice with 5 ml prewarmed (37°C) medium without Cd, lysed in 10 ml distilled water, and frozen and thawed twice. The cell lysate was centrifuged at 2,000g for 10 min and the supernatant chromatographed on a Sephadex G-75 column (K 16/100) 1.6×79 cm in Tris-HCl buffer 0.001 M, pH 8.6.

The column was calibrated with bovine albumin, trypsin inhibitor soyabean protein and cytochrome *c*. The result of gel filtration of the lysate of HE cells resistant to $100\ \mu\text{M}$ Cd grown in the presence of ^{109}Cd is shown in Fig. 2. The elution volume for peak 2 was the same as for cytochrome *c* and corresponds to a molecular weight of about 12,000.

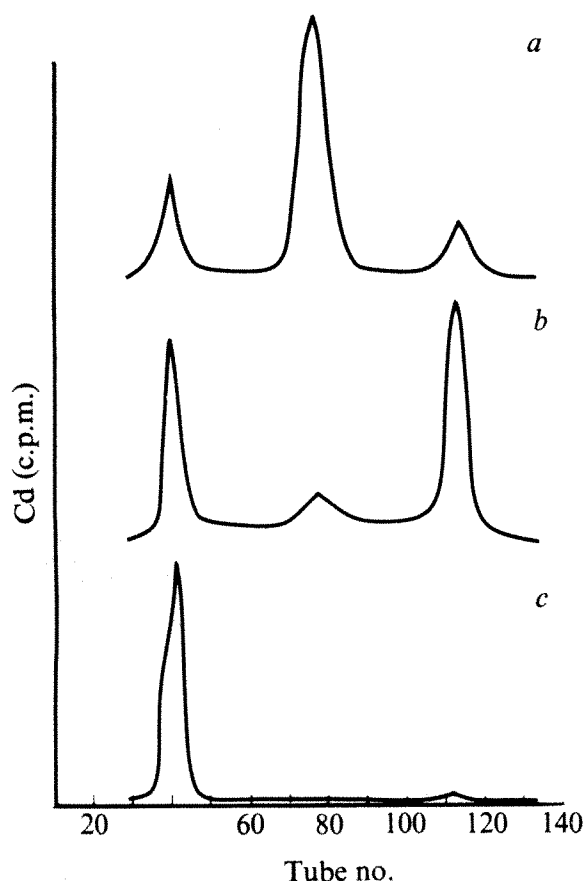


Fig. 2 Gel filtration (Sephadex G-75) of lysate of: *a*, human skin epithelial cells made resistant to $100\ \mu\text{M}$ Cd; *b*, cells not resistant to Cd; *c*, growth medium (Dulbecco's modification of Eagles' medium supplemented with 17.5% serum and with $1.3\ \mu\text{M}$ ^{109}Cd). Tube 40 represents the exclusion volume (molecular weight about 70,000), tube 80 corresponds to a molecular weight of about 12,000; tube 120 is the total volume of the column and corresponds to a molecular weight of less than 1,000–3,000.

Figure 2 also shows the result of gel filtration of lysate of HE cells not resistant to Cd also grown for 24 h in the presence of $1.3\ \mu\text{M}$ ^{109}Cd . No peaks corresponding to CdBP of molecular weight about 12,000 could be detected. Growth medium likewise did not contain any CdBP with a molecular weight of 12,000. A Cd peak was demonstrated corresponding to a protein eluted with the void volume, which means that its molecular weight is above 70,000. Both types of cell had a Cd peak corresponding to the total volume of the column, that is Cd conjugated to some compound with a molecular weight of less than 1,000–3,000. HE cells resistant to $100\ \mu\text{M}$ Cd, but grown without any Cd added to the medium for 4 weeks, also contain a Cd-binding macromolecule with a molecular weight of 12,000. After about 20 generations in Cd-free medium these cells were still resistant to $100\ \mu\text{M}$ Cd and they also still had CdBP with a molecular weight of 12,000. We do not know,

however, whether minute amounts of Cd were still present in the cells at that time. In some experiments Cd isotope was added to the medium and the cell lysate after collection of the cells. The results were essentially the same; CdBP of molecular weight about 12,000 could be demonstrated in lysates from Cd-resistant cells, but not in the growth medium or in HE cells not exposed to Cd.

In these experiments we have thus found a good correlation between Cd resistance and the appearance of CdBP. Since our epithelial cells have been derived from human skin, these experiments show that CdBP may be produced in organs other than liver and kidney. Two different cadmium-binding proteins (metallothioneins) have been purified from human livers by Bühler and Kägi⁶. They determined the molecular weights to be about 6,100 for the peptide moiety. Nordberg *et al.*⁸ isolated and described metallothionein from rabbit with a molecular weight of about 6,000. Weser *et al.*⁹ found, however, a molecular weight of about 12,000 for rat and chicken. They discuss whether their protein might be a dimer of the rabbit protein isolated by Nordberg *et al.*⁸. The protein which seems to develop parallel to the resistance to cadmium in the cells used by us may constitute a dimer of the protein described by Bühler and Kägi⁷. Kägi and Vallee¹⁰ previously described an equine protein containing cadmium and zinc with a molecular weight of about 10,000, but they do not discuss the possibility of dimer generation.

We believe that these cells are well suited for the study of induction of cadmium-binding proteins and their biological role, including the possible correlation between Cd resistance and content of Cd-binding proteins.

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Ricin resistance in baby hamster kidney cells

LITTLE is understood about the function of the complex oligosaccharide side chains present in cell membrane glycoproteins. One approach to this problem has been to select from cultured mammalian cells variants deficient in cell surface binding sites for certain plant lectins, since in these cells some part of the membrane glycoproteins' carbohydrate moieties should be missing or altered. Such variants have been isolated from Chinese hamster ovary (CHO) cells using ricin¹ (from *Ricinus communis* seeds) and phytohaemagglutinin² (from *Phaseolus vulgaris*), cytotoxic lectins which bind to membrane glycoprotein galactose and N-acetylgalactosamine groups^{3–5}.

We describe here 22 clones of the baby hamster kidney cell line, BHK 21 C13 (ref. 6) resistant to ricin, several of which seem to be deficient in cell surface ricin binding sites.

In our studies BHK cells (approximately 2×10^5) growing in Glasgow modified minimal essential medium (MEM) plus 10% foetal bovine serum (FBS) at 33°C on 60-mm Falcon plastic dishes were treated with methyl-N-nitro-N-nitrosoguanidine

(0.5 $\mu\text{g ml}^{-1}$) for 1 d. Surviving cells (about 28% of the total) were grown for a further 4 d to establish mutant phenotypes, trypsinised and replated at 5×10^5 cells per dish in MEM plus 2% FBS. Since FBS contains ricin-binding glycoproteins the lower concentration was used in the selection procedure. The cells were grown to approximately 3×10^6 per dish at 39 °C, ricin (0.2 μg or 1 $\mu\text{g ml}^{-1}$) was added and incubation continued for 7–10 d in the presence of ricin with the medium changed every 3 d. In some experiments the ricin concentration was increased from 0.2 μg to 1 $\mu\text{g ml}^{-1}$ after the first 3 d. Many

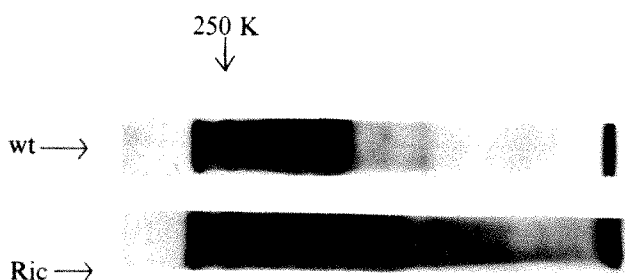
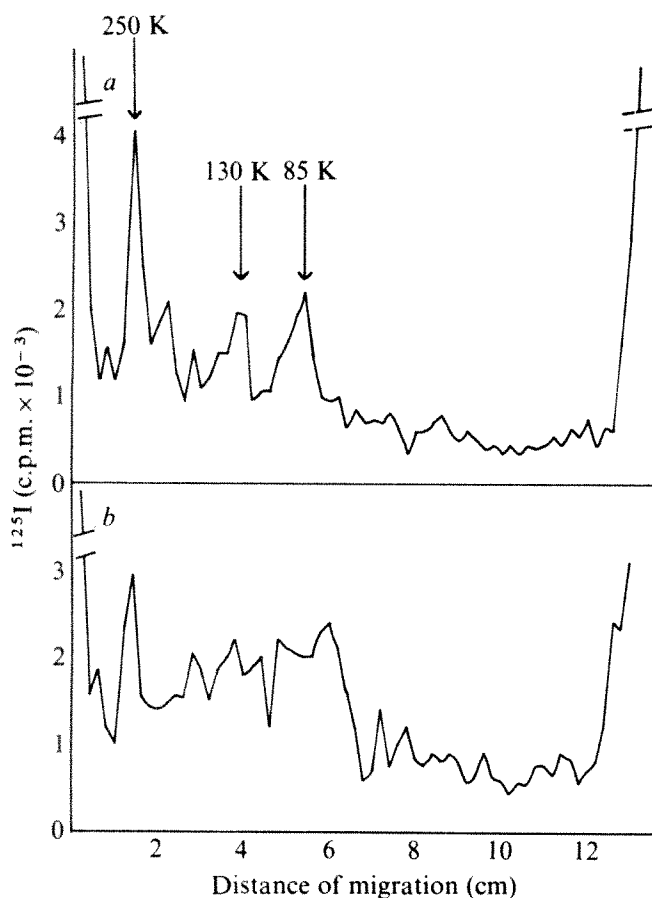


Fig. 1 Lactoperoxidase-catalysed surface iodination of baby hamster kidney (BHK) cells. Intact cells of normal BHK cells and the ricin resistant variant Ric^R14 were iodinated with ¹²⁵I by standard lactoperoxidase labelling techniques. The iodinated cells were dissolved in hot sodium dodecyl sulphate plus 2-mercaptoethanol and examined by slab gel polyacrylamide gel electrophoresis. The radioiodinated bands represent surface components accessible to lactoperoxidase, an enzyme that does not penetrate the plasma membrane of intact cells to label cytoplasmic proteins or glycoproteins. The bands were detected (top panel) by autoradiography or by (lower panel) cutting up the gel into 2-mm slices and counting in a Wallac gamma spectrometer. Approximate molecular weights of proteins were estimated by reference to the mobilities of reovirus structural proteins¹⁵. *a*, Normal cells at confluence; *b*, ricin-resistant cells Ric^R14 at high cell density (confluence). Note the appearance of a major iodinated band of apparent molecular weight 250,000 in the normal and Ric^R14 cells at confluence. Note the enhanced mobility of the other iodinated bands present in Ric^R14 cells relative to the corresponding iodinated components of the normal BHK cells.

Table 1 Selection, resistance and ricin binding sites of ricin-resistant BHK cell clones

Cells	Ricin concentration for selection	Relative plating* efficiency (± ricin)%	Relative binding† of ¹²⁵ I-ricin
Normal	—	1–2	1.0
Ric ^R 1	0.2 $\mu\text{g ml}^{-1}$	35	1.12
2	0.2 $\mu\text{g ml}^{-1}$	25	1.12
3	0.2 $\mu\text{g ml}^{-1}$	2.5	ND
4	0.2 $\mu\text{g ml}^{-1}$	ND	ND
5	0.2 $\mu\text{g ml}^{-1}$	17	ND
6	1 $\mu\text{g ml}^{-1}$	60	0.15
7	1 $\mu\text{g ml}^{-1}$	83	0.43
8	1 $\mu\text{g ml}^{-1}$	91	ND
9	1 $\mu\text{g ml}^{-1}$	50	0.12
10	1 $\mu\text{g ml}^{-1}$	40	0.13
11	1 $\mu\text{g ml}^{-1}$	83	ND
12	1 $\mu\text{g ml}^{-1}$	43	1.36
13	1 $\mu\text{g ml}^{-1}$	75	ND
14	0.2 $\mu\text{g ml}^{-1}$	43	0.12
15	then 1.0 $\mu\text{g ml}^{-1}$	107	0.14
16	1.0 $\mu\text{g ml}^{-1}$	50	1.05
17	1.0 $\mu\text{g ml}^{-1}$	35	0.27
18	1.0 $\mu\text{g ml}^{-1}$	70	0.47
19	1.0 $\mu\text{g ml}^{-1}$	70	1.97
20	1.0 $\mu\text{g ml}^{-1}$	51	0.19
21	1.0 $\mu\text{g ml}^{-1}$	83	0.10
22	0.2 $\mu\text{g ml}^{-1}$	40	1.5
	then 0.5 $\mu\text{g ml}^{-1}$		

* Trypsinised cells in Ham's F10 medium plus 10% foetal calf serum were plated on 60-mm Falcon dishes at approximately 1,000 cells per dish. After 1–2 h at 39 °C ricin was added at 0.1 $\mu\text{g ml}^{-1}$ final concentration and after further incubation for 7–10 d, cell colonies were stained with 1% gentian violet in 20% ethanol and counted. The relative plating efficiency is the number of colonies developed in the presence of ricin compared with the number of colonies developed in the absence of ricin from equal numbers of cells.

† Cells growing on 35-mm diameter plastic dishes were rinsed with warm phosphate-buffered saline. Serial dilutions of a solution of lactoperoxidase-catalysed, ¹²⁵I-labelled lectin (usually at least 10⁷ c.p.m. mg⁻¹ of protein) were added (each 1 ml). After incubation at room temperature for 60 min the cells were rinsed several times to remove unbound lectin, dissolved in alkali and assayed for cell protein and ¹²⁵I radioactivity. Binding curves were treated as Scatchard plots to obtain the total number of binding sites per cell. Values are expressed relative to normal BHK cells. The number of ricin binding sites for normal BHK cells is 5.6×10^6 per cell.

cells detached from the plastic surface and were removed during the changes of medium. Colonies surviving at a frequency of approximately 10⁻⁶ in ricin (1 $\mu\text{g ml}^{-1}$) were removed by trypsinisation under sterile glass cloning cylinders, transferred to Falcon dishes containing ricin-free fresh Ham's F10 medium containing 10% FBS, and grown to high densities to establish cell lines which thereafter were cultured in MEM plus 10% FBS. No surviving colonies were obtained from non-mutagenised cells treated with ricin at 1 $\mu\text{g ml}^{-1}$.

All of our clones except one (Ric^R3) retain a substantial degree of their resistance when cultured in the absence of ricin (Table 1), even for 1 yr, and therefore exhibit stable heritable alterations. Resistance to ricin is expressed equally at both 33 °C and 39 °C, and thus no clone exhibits temperature-sensitive resistance, which we had hoped our selection method would provide. Of the 22 resistant clones several of those tested, for example Ric^R1, 2, 12, 16, 19, bind about as much or more ricin as do normal sensitive BHK cells (Table 1). It is likely that in these cells resistance is mediated by either a lack of endocytosis of bound lectin⁷ or an inability of the lectin once in the cytoplasm to affect protein synthesis^{8,9}. Other resistant clones, however, are deficient (Table 1) in ricin binding sites, for example, Ric^R9, 14, 15, 21.

Ricin-resistant clones deficient in lectin binding sites are probably defective in biosynthetic steps involved in their assembly. An example of such a step is the addition of N-acetylglucosaminyl residues to the internal mannose core of growing oligosaccharide side chains of surface membrane glycoproteins terminating in the sequence sialyl-galactosyl-N-acetylgluco-

saminyl-mannose¹⁰. Clones of CHO cells deficient in binding sites for phytohaemagglutinin and ricin, respectively, are deficient in a β -N-acetylglucosaminyl transferase^{11,12}, and presumably cannot transfer N-acetylglucosamine or the penultimate galactose to those glycoprotein oligosaccharides necessary for lectin binding. Enzymatic analysis of cell extracts of some of our ricin-resistant BHK clones which are deficient in binding sites, that is Ric^R14, 15, 18 and 21, has indicated that Ric^R14, but not the others, has less than 10% of normal β -N-acetylglucosaminyl transferase activity when neuraminidase, β -galactosidase and β -N-acetylglucosaminidase-treated α_1 -acid glycoprotein is used as acceptor¹³. The activities of sialyl and galactosyl transferases in all these clones, including Ric^R14, assayed using the appropriate derivatives of the α_1 -acid glycoprotein are similar to normal BHK cells. It is possible, of course, that small differences in specific glycosyl transferases, for example, a galactosyl transferase, cannot be detected or that α_1 -acid glycoprotein derivatives are not suitable acceptors for all transferases, or simply that the biosynthetic block lies elsewhere, for example, N-acetylgalactosaminyl transferase.

Additional evidence that our ricin-resistant clones deficient in binding sites are blocked at some stage of carbohydrate chain assembly comes from surface labelling. Several surface components of normal BHK cells are labelled by ¹²⁵I-iodination catalysed by lactoperoxidase (Fig. 1). In clones Ric^R14 (Fig. 1), Ric^R7, Ric^R15 and possibly Ric^R21 (not shown) the radioactive bands move more rapidly during polyacrylamide gel electrophoresis than the radioactive bands of normal cells, which is consistent with the surface glycoproteins of these clones being less well glycosylated. In contrast, the surface glycoproteins of Ric^R16 and Ric^R19, resistant clones not deficient in ricin binding sites (Table 1), show no increase in migration on polyacrylamide gels (R.N., unpublished). It is interesting that the large iodinated glycoprotein of apparent molecular weight 250,000, described by others in various cells¹⁴, is present in normal BHK cells and in the Ric^R14 clone (Fig. 1) when the cells are at high density, and that the mobility of this large glycoprotein apparently does not change in the ricin-resistant clone. Either this large glycoprotein does not bind ricin and is unaffected by the glycosylation defect, or the carbohydrate is a minor part of the whole molecule and decreased glycosylation is not detected by our method.

Further evidence for premature termination of glycoprotein oligosaccharides in our clones deficient in ricin binding sites comes from binding studies with the lectin concanavalin A (con A) with specificity for mannosides. In clones Ric^R14, 15 and 21 the number of ¹²⁵I-con A binding sites is increased 1.5–3.0-fold, suggesting increased exposure of terminal mannose groups.

We conclude therefore that stable alterations in surface glycoprotein oligosaccharides can be selected for in BHK cells. We have isolated clones which bind ricin poorly due presumably to a loss of surface galactose or N-acetylgalactosamine, and in one case (Ric^R14) we have demonstrated an enzyme deficiency in a β -N-acetylglucosaminyl transferase which could lead to such a loss. The alterations to surface glycoprotein oligosaccharides seem, however, to have negligible effects on the normal growth processes of our variants. Nevertheless, the availability of stable variants of BHK cells showing altered carbohydrate structure will be useful in studies of the role of surface carbohydrates in intercellular properties such as adhesion—several of our variant cells, particularly Ric^R14, show weaker adhesion to plastic culture surfaces—and metabolic cooperation.

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Liquid-crystalline characteristics of the thick filament lattice of striated muscle

THE lattice of thick filaments (A-band lattice) in striated muscle is similar in many respects to smectic liquid-crystalline structures in which macromolecules are arranged parallel to and equidistant from each other forming lattice planes^{1,2}. Like other liquid-crystalline systems, this lattice may exist in two characteristic conditions which are determined either by a simple balance between electrical forces^{3–5,7} or subject to an additional volume-limiting force^{6–8}. Some mathematical analyses^{2,4–6,9–12} agree with the experimental results obtained

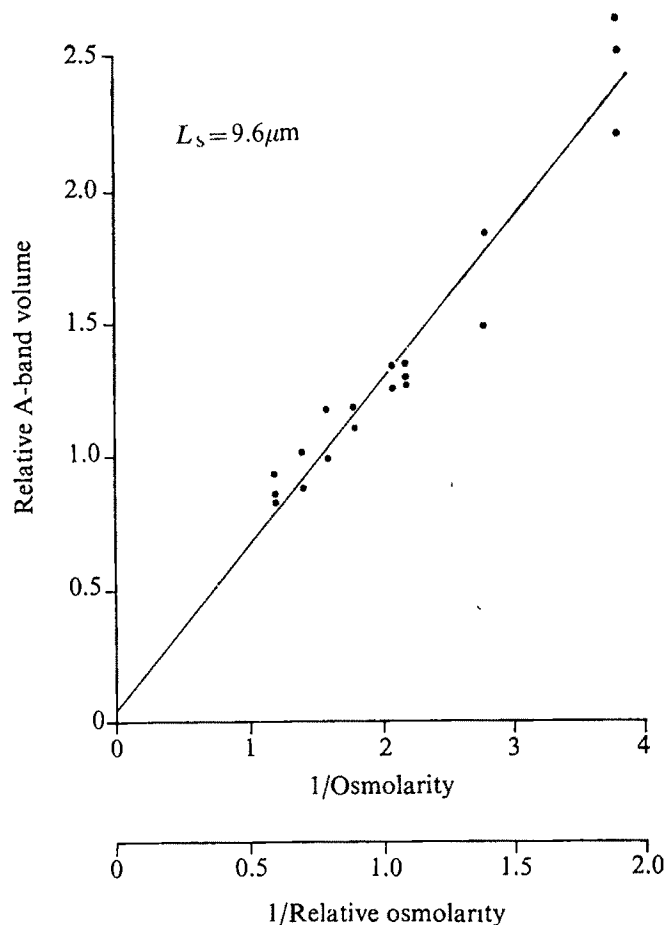


Fig. 1 A-band volume of muscle as a function of osmolarity. The relative volume of the A-band in living crayfish muscle fibres is plotted against the reciprocal osmolarity of sodium chloride media. The A-band volume across the whole fibre was determined from the optically measured diameter of the fibre at a constant sarcomere length and the dimensions of the thick filaments (4.4 μ m). The osmolarity was varied by adjusting the NaCl concentration of the normal medium (NaCl, 200 mmol l⁻¹; CaCl₂, 13.5 mmol l⁻¹; KCl, 5 mmol l⁻¹; Tris, 5 mmol l⁻¹, buffered to pH 7.4).

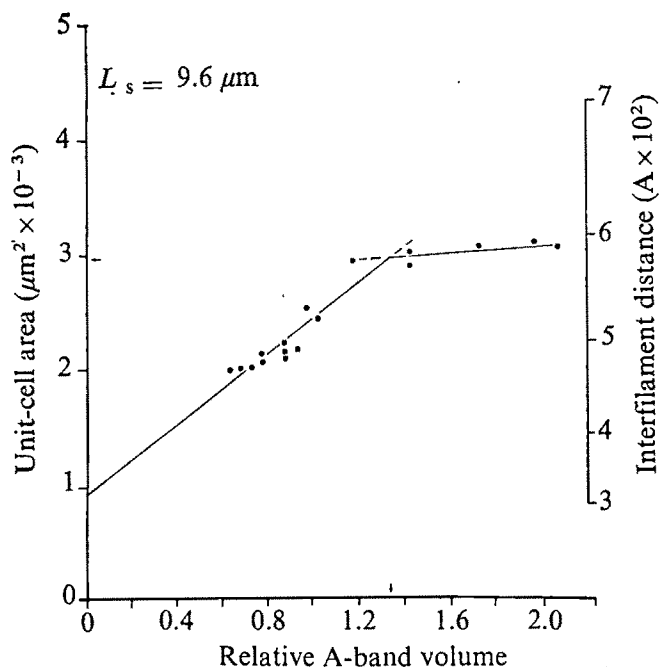


Fig. 2 Unit-cell area of the thick filament lattice of intact fibres as a function of the A-band volume. The unit-cell area and interfilament distance of the thick filament lattice of crayfish muscle at constant sarcomere length is plotted against the relative A-band volume as determined by the osmolarity of the sodium chloride media (compare Fig. 1). The close approximation of the data to a straight line (fitted by the method of least squares, $r = 0.92$) over a portion of the range indicates a region in which the lattice exhibits the Boyle-van't Hoff pressure-volume relationship of the fibre. In this region the lattice behaviour is similar to that of the volume-constrained liquid-crystal. The ordinate intercept indicates an osmotic dead space. The break in the line at an A-band volume of 135% of the control demonstrates that the unit-cell area of the thick filament lattice no longer follows the A-band volume, indicating that the lattice changes from a volume-constrained to an electrically balanced condition when the thick filament separation reaches approximately 582 Å at a sarcomere length of 9.6 μm .

from glycerinated muscle³ and skinned fibres¹³⁻¹⁵, but are only applicable to the electrically balanced liquid-crystalline condition. The behaviour of the thick filament lattice in intact living muscle cannot be explained exclusively in terms of a balance between Van der Waals' and electrostatic force⁵ because the Donnan-osmotic steady state across the sarcolemma determines the net water flux and thereby controls the fibre and lattice volumes. Cross bridges or M-line bridges cannot be evoked as a mechanism for maintaining the lattice regularity because the array persists in EGTA-relaxed skinned fibres in muscles having no M-line structure¹⁴.

The volume of a single muscle fibre of crayfish (*Orconectes*), and in particular the A-band volume, is a function of the osmolarity of the bathing medium. This is evident from Fig. 1, in which the relative A-band volume across the fibre (optically determined fibre diameter times thick filament length) is plotted against the reciprocal of the osmolarity of the medium. The approximation of the data to a straight line (fitted by the method of least squares, $r = 0.97$) indicates compliance with the Boyle-van't Hoff pressure-volume relationship. In physiological conditions with the usual complement of myofilaments, the fibre volume is less than that necessary for the attainment of a balance between Van der Waals' attractive and Coulombic repulsive forces. The unit-cell volume of the sarcomere (the unit-cell volume of the thick filament lattice extended to the Z-lines) and the thick filament separation of the intact fibre also follows the Boyle-van't Hoff relationship exhibited by the whole fibre¹⁵. Figure 2 shows that the thick filament spacing is a function of A-band volume.

The unit-cell area of the A-band lattice (determined from the 1, 0 X-ray diffraction reflections of single fibres at constant sarcomere length) is plotted against the A-band volume of the whole fibre (determined by the osmolarity of the medium and measured optically). The linear fit ($r = 0.92$) of the data signifies agreement with the Boyle-van't Hoff equation and the ordinate intercept is indicative of an osmotic dead space¹⁵. These data are consistent with volume-constrained liquid-crystalline behaviour in which elongate particles comprising the lattice can be confined to a volume smaller than that which is necessary for an electrical balance¹⁶. Thus, interaxial distance between the particles (d) is inversely proportional to the square root of the particle concentration $[P]$:

$$d \propto 1/\sqrt{[P]} \quad (1)$$

Since particle concentration is inversely proportional to the volume, in the case of muscle the filament spacing within the living fibre is a function of the A-band volume (V_A) to which the thick filaments are confined by the smectic nature of the lattice:

$$d \propto \sqrt{V_A} \quad (2)$$

The explanation is straightforward. When interaxial separation is decreased by reduction of the available volume, the electrostatic repulsive forces which vary exponentially with separation⁹ are enhanced and must necessarily become greater than the Van der Waals' forces which vary inversely as the fifth power of distance⁹. Thus, the interaction energy of the system is

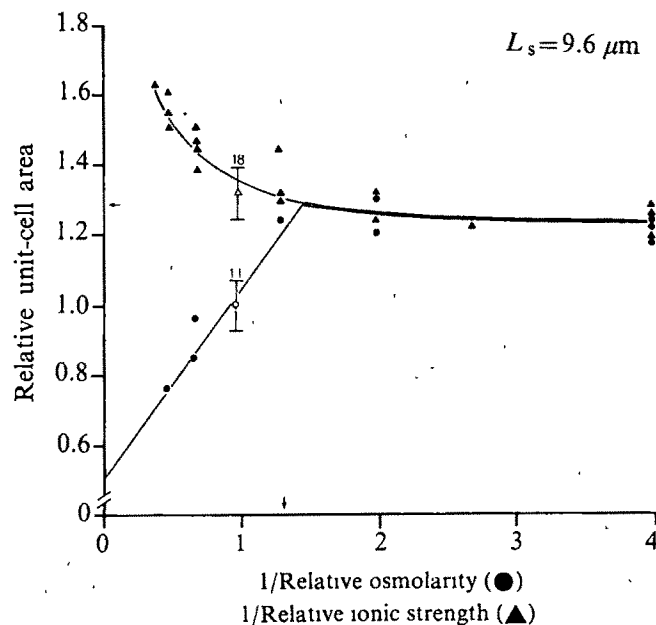


Fig. 3 Comparison of intact and skinned fibre lattice behaviour, recomputed and redrawn from April *et al.*¹⁵. The relative unit-cell areas of fibres in potassium propionate media are plotted against the reciprocal of the relative osmolarity for intact fibres (●) and the corresponding ionic strength for skinned fibres (▲). The lines are fitted by the method of least squares ($r_s = 0.94$; $r_{\Delta} = 0.90$). The open symbols represent mean values with the number of experiments and the standard deviations from the mean indicated. The divergent behaviour of the two lattices below a relative inverse osmolarity (or corresponding A-band volume) of approximately 130% of the control signifies different liquid-crystalline conditions. Above 130%, the behaviour of the intact fibre lattice is similar to that of the skinned fibre, indicating a change in the lattice of the intact fibre from a volume-constrained to an electrically balanced liquid-crystalline condition.

increased. If additional volume becomes available, the filaments move apart uniformly with the additional interaction energy (increased electrostatic forces) responsible for this readjustment. When interfilament distance increases to the point where opposing electrical forces balance, the interaction energy is minimal and further movement ceases unless pH or ionic strength of the medium is altered^{3,7,13,15}.

In other words, the lattice expands to fill the A-band until an equilibrium between Van der Waals' and electrostatic forces is reached.

The volume-limiting effect of the sarcolemma in the living fibre is equivalent to the performance of osmotic work whereby the interaction energy of the system is increased through confinement of the thick filaments to a volume less than that necessary for electrical force balance. It should be noted that in the volume-constrained condition the osmotic pressure counterbalances the electrostatic repulsive forces and relegates the Van der Waals' forces to a minor role in the maintenance of lattice stability. Thus, in the volume-constrained condition representative of living muscle, the fibre volume determines the maximum possible thick filament separation and, thus, the magnitude of the electrostatic forces. Within this confine the electrostatic forces between the thick filaments maintain the hexagonal lattice structure by mutual repulsion.

The volume-constrained liquid-crystalline condition of living single muscle fibres and the electrically balanced liquid-crystalline condition of skinned fibres can be compared in Fig. 3, in which the relative unit-cell areas of the lattices are plotted against the relative osmolarity and corresponding ionic strength of the bathing medium at pH 7.4. Both preparations were in identical media and the change in osmolarity or ionic strength was accomplished by adjusting the potassium propionate concentration of the relaxing solution. Clearly the behaviours of the filament lattices are different. The interaxial spacing in the skinned fibre is sensitive to the ionic strength of the medium whereas the interaxial spacing of the intact fibre is sensitive only to osmotically induced volume changes. In addition, on removal of the sarcolemma from a living fibre in conditions where the external ionic strength matches that of the sarcoplasm, the lattice immediately expands to the dimensions observed for the electrically balanced lattice at that particular ionic strength and pH. This indicates that the physiological volume of the living muscle fibre is inadequate for an equilibrium between van der Waals' and electrostatic forces and that such a condition is achieved after skinning the fibre.

Another inference from the data presented in Figs 2 and 3 is that as the intact fibre swells a point is reached where the filament lattice ceases to expand while the fibre as a whole continues to swell. This point represents a transition from a volume-constrained to an electrically balanced liquid-crystalline lattice because, once this point is reached, the lattice no longer exhibits a volume dependence and the behaviour becomes similar to that of the skinned fibre with respect to changes in ionic strength. Thus by sufficient swelling the volume constraint on the lattice can be removed. From this it follows that the spacing of the lattice in the intact fibre is determined by the condition that it fills the available volume until the spacing is sufficient for Van der Waals' attractions to balance electrostatic repulsion. Consequently, the previous calculations by Elliott *et al.*⁵, from which they concluded that lattice spacing was determined solely by the balance of Van der Waals' and electrostatic forces, cannot be applied to living muscle in physiological solution and their explanation for constant-volume shortening must be incorrect.

The principal conclusion to be drawn is that the thick filament lattice of living muscle is normally constrained and is sensitive only to changes in A-band volume. In contrast, the lattice state of glycerinated muscle, skinned fibres and greatly swollen fibres is electrically balanced *vis-à-vis* van der Waals' attractive and electrostatic repulsive forces and is sensitive to any parameter which affects electrostatic charges.

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Low doses of DNP-D-GL, a potent hapten-specific tolerogen, are immunogenic *in vitro*

THE DNP conjugate of a D-amino acid copolymer of glutamic acid and lysine (DNP-D-GL) is a potent inducer of DNP-specific tolerance *in vivo* and *in vitro*^{1,2}. Simultaneous administration of DNP-D-GL and allogeneic cells to mice, however, results in anti-DNP antibody formation rather than tolerance³. DNP-D-GL thus seems to block activation of DNP-specific precursor cells in ordinary circumstances but to activate cells altered by allogeneic effect factors⁴. We have attempted to define further the conditions in which DNP-D-GL can induce antibody formation, a question made more relevant by the proposal that nucleic acids coupled to D-GL may be useful in preventing autoimmune disease, a situation where induction of antibody formation rather than tolerance would be unfortunate.

We have investigated the conditions in which DNP-D-GL can act as a tolerogen or an immunogen for T-independent anti-DNP responses of mouse spleen cells *in vitro*. Nanogram quantities of DNP-D-GL induced anti-DNP antibody formation, while larger amounts prevented responses to DNP-conjugates, as previously reported². Presentation of the DNP-D-GL in the form of antigen-pulsed macrophages further increased its immunogenicity and (or) decreased its tolerogenic potential. DNP-D-GL-induced anti-DNP antibody in nude mouse spleen

Table 1 *In vitro* anti-DNP PFC response of nu/nu and nu/+ spleen cells to DNP₇₅-D-GL

DNP ₇₅ -D-GL ($\mu\text{g ml}^{-1}$)	Mean anti-DNP IgM PFC per culture \pm s.e.*	
	nu/nu spleen cells	nu/+ spleen cells
10^{-6}	25 \pm 8	68 \pm 29
10^{-5}	167 \pm 23	87 \pm 24
10^{-4}	189 \pm 42	152 \pm 38
10^{-3}	291 \pm 99	272 \pm 46
10^{-2}	262 \pm 63	96 \pm 54
10^{-1}	137 \pm 30	0
10^0	0	0
10^{-2} DNP ₃₉ -AECM-Ficoll	1,023 \pm 291	1,025 \pm 98
SRBC	25 \pm 5	890 \pm 90

*Numbers are the mean PFC specific for DNP (background subtracted) in three replicate experiments \pm s.e. Each experiment used spleen cells from one nu/nu and one nu/+ animal.

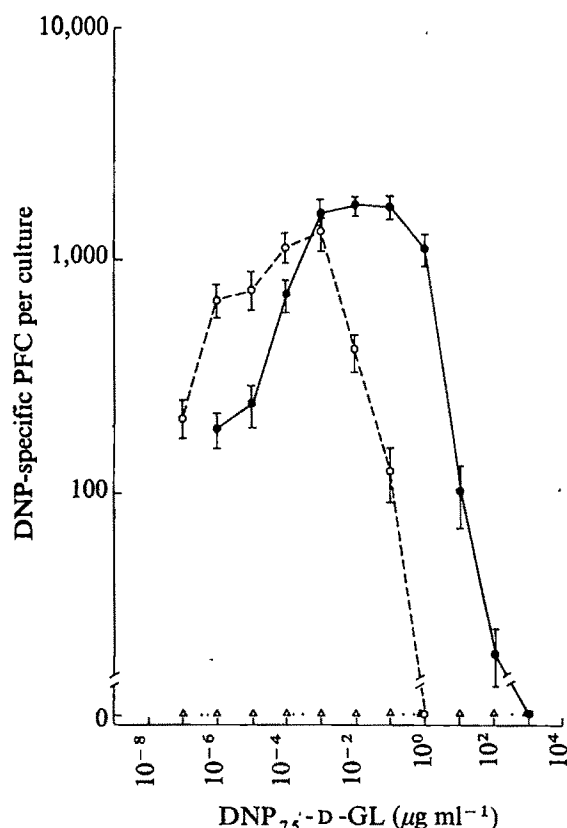


Fig. 1 The anti-DNP antibody response of BALB/c spleen cells cultured *in vitro* with DNP₇₅-D-GL. ○, Continuous DNP-D-GL added to normal spleen cells; ●, spleen cells exposed to indicated concentration of DNP-D-GL, washed, and antigen-pulsed adherent cells prepared. Normal non-adherent cells were added to the DNP-D-GL-pulsed adherent cells. △, Continuous DNP-D-GL added to macrophage-depleted non-adherent cells.

cell cultures. We conclude that DNP-D-GL is not intrinsically tolerogenic but can deliver both activating and inactivating signals to B lymphocytes, and that it is the concentration of DNP-D-GL and (or) the form of presentation which determines which of the two signals is dominant.

Spleen cells from BALB/c mice or from NIH outbred homozygous and heterozygous nude mice were cultured in Mishell-Dutton conditions as previously described⁶. DNP₇₅-D-GL (75 mol DNP per 105,000 daltons average molecular weight of D-GL, G:L ratio of 60:40) was added to cultures to give final concentrations from 10^{-8} to 10^4 $\mu\text{g ml}^{-1}$. DNP-D-GL-pulsed macrophages were prepared by incubating spleen cells with antigen for 1 h at 4 °C, washing three times, plating in culture dishes for 1 h at 37 °C, and washing the adherent cells twice more. Direct plaque-forming cells (PFC) were counted using TNP-coupled sheep erythrocytes⁶ as indicator cells. Induction of DNP-specific tolerance *in vitro* was assayed by the simultaneous addition of 10^{-3} $\mu\text{g ml}^{-1}$ DNP₃₉-aminoethylcarbonyl-methyl (AECM)-Ficoll, a thymus-independent antigen *in vitro*⁶. Both DNP₇₅-D-GL and DNP₃₉-AECM-Ficoll were endotoxin-free by the *Limulus* amoebocyte lysate gelation assay⁷. Specificity of tolerance induction was shown by lack of depression of *in vitro* responses to sheep erythrocytes.

Figure 1 shows the mean anti-DNP response in three replicate experiments of spleen cells cultured with DNP₇₅-D-GL in three conditions: soluble antigen presented continuously to normal spleen cells; antigen-pulsed adherent cells (>90% macrophages) mixed in a 1:20 ratio with normal non-adherent (macrophage-depleted) spleen cells; or soluble antigen added directly to non-adherent cells. Soluble DNP-D-GL induced substantial anti-DNP-PFC on the part of whole spleen cell populations but failed to induce any detectable responses in non-adherent spleen cells. DNP-D-GL pulsed macrophages,

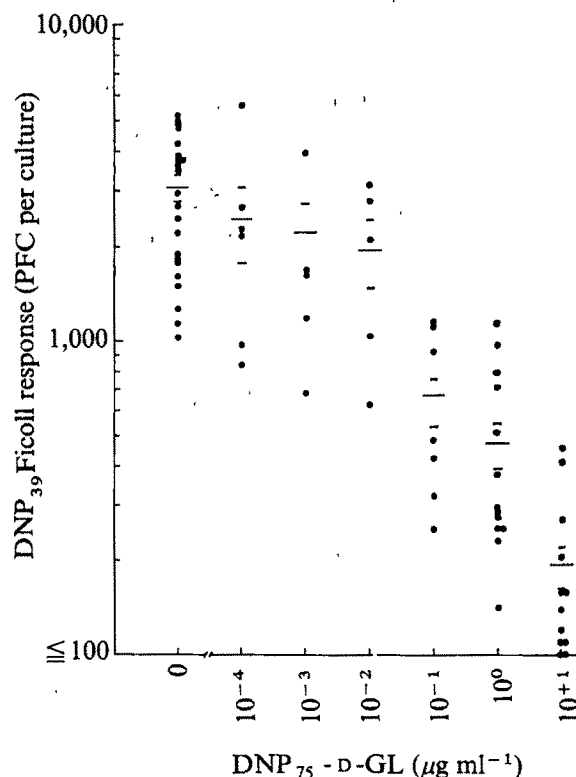
however, stimulated quite marked anti-DNP responses by non-adherent spleen cells. Since the amount of antigen retained by pulsed adherent cells was 1% or less, of the original dose indicated in Fig. 1 (as determined by retention of ³H-DNP-D-GL), the optimal amount of DNP₇₅-D-GL for inducing antibody production was 10^{-3} – 10^{-4} $\mu\text{g ml}^{-1}$ for both soluble antigen and pulsed macrophages.

The effect of immunogenic and suprainmunogenic concentrations of DNP-D-GL on the anti-DNP response of normal spleen cells to DNP₃₉-AECM-Ficoll is shown in Fig. 2. Data are pooled from 22 experiments with each point representing a group mean response in one experiment; the geometric mean \pm s.e. is plotted. Immunogenic concentrations of DNP-D-GL (10^{-4} – 10^{-3} $\mu\text{g ml}^{-1}$) caused an insignificant reduction in the DNP-Ficoll response, but higher concentrations were highly suppressive of anti-DNP responses. This effect was antigen-specific in that parallel control responses to sheep red blood cells were not reduced by DNP-D-GL.

Spleen cells from individual outbred athymic nude mice responded to DNP-D-GL *in vitro* as well as spleen cells from phenotypically normal littermates (Table 1), although both had low anti-DNP PFC responses compared with BALB/c spleen cells (for example, Fig. 1). Nude spleen cells were susceptible to tolerance induction by DNP-D-GL; for example, $1 \mu\text{g ml}^{-1}$ reduced the DNP-Ficoll response from 1,204 to 240 PFC per culture. Induction of both antibody synthesis and DNP-specific tolerance thus seem to occur in mice lacking detectable T cell function.

These results indicate that DNP-D-GL can act as a T-independent antigen as well as a T-independent tolerogen in normal and nude mice. The response to DNP-D-GL does not occur when macrophage numbers are diminished, but it is not yet clear whether the remaining non-adherent lymphocytes are unable to respond to DNP-D-GL or are rendered tolerant by continuous exposure to soluble antigen. We conclude that the

Fig. 2 Inhibition of the anti-DNP response of BALB/c spleen cells to DNP₃₉-AECM-Ficoll by various concentrations of continuous DNP₇₅-D-GL. Each point is the mean response in a single experiment; data are pooled from 22 experiments. Horizontal bars indicate the geometric mean \pm s.e.



poorly metabolisable nature of D-Gl does not make it an obligate tolerogen, at least for normal mouse spleen cells in culture, and that its mechanism of tolerance induction may serve as a useful model for naturally occurring tolerance to antigens such as polysaccharides which display T-independent-like characteristics.

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Continuous production of peroxidase, esterase, alkaline phosphatase and lysozyme by clones of promyelocytes

THE classic description of maturation of cells of the bone marrow myeloid series is based on static observation of morphological and biochemical differences between cells in close proximity¹. It is generally accepted that multipotential stem cells progress under appropriate leukopoietic stimuli to myeloblasts, and then to promyelocytes, myelocytes, metamyelocytes and at least three specific mature populations with distinct biological functions: polymorphonuclear neutrophils, basophils and eosinophils². The stage at which a cell becomes committed to a specific population is unknown. One approach to defining the maturation relationship between myeloid cells has been through study of the production of enzymes³⁻⁵, chemical mediators⁶ and other intracellular or surface proteins⁶⁻⁸ specific to each leukocyte population. Histochemical techniques for identification of myeloid enzyme production have been used to correlate the production of these proteins with different stages of morphological maturation¹⁰. The inability to establish tissue culture lines derived from single myeloid cells stably arrested in development has prevented study of the degree of differentiation of cells in each

stage of maturation. We report here the establishment of multiple clones of rat myeloid cells which maintain stable promyelocyte morphology *in vitro* and produce four enzymes. Clones derived from more primitive myeloblast cells produce only lysozyme.

The Jones chloroma¹¹, a rat leukaemia line passaged more than 50 times *in vivo*, has maintained morphology typical of normal promyelocytes. Bone marrow samples obtained from affected rats are replaced with leukaemic cells which are positive in assays for esterase, alkaline phosphatase, peroxidase and lysozyme; however, fresh leukaemia cell explants or cells grown for short periods in tissue culture demonstrate enzyme positivity in only a fraction of cells tested by each histochemical assay (unpublished results). Similar observations are common when marrow is examined from a wide variety of experimental and human myelogenous leukaemias. This could reflect stable differences in enzyme production by different subpopulations within a leukaemia line or growth cycle-dependent variation in enzyme production by a truly homogenous population of cells. Another leukaemia line, the Wistar/Furth (W/Fu) acute myelogenous leukaemia (AML), has been shown to exhibit more primitive morphology and produce only lysozyme *in vivo* and *in vitro*¹². Here, 5-10% of cells consistently have seemed lysozyme-negative and could represent a subpopulation arrested in a developmental stage before that associated with lysozyme production.

To test these possibilities clonal lines were established in tissue culture from each transplantable tumour. These were maintained *in vitro* for around 2 months and then tested both for enzyme production and for morphological appearance. As Table 1 shows, each of 23 clones of the Jones chloroleukaemia produced peroxidase, esterase, alkaline phosphatase and lysozyme. Fifteen clones of W/Fu AML cells were indistinguishable in myeloblast morphology and production of a single enzyme, lysozyme. The proportion of positive cells in each assay did not differ significantly between clones tested.

Each clone of either line had a similar *in vitro* doubling time of 15-20 h, and saturation density of 3.0×10^6 - 4.0×10^6 cells ml⁻¹ in liquid medium. The clonal lines formed colonies with an efficiency of 5.0-5.5% in either liquid medium or semi-solid medium containing 0.9% methylcellulose. These *in vitro* growth parameters were indistinguishable from those of the parent lines.

The clonal lines were passaged continuously and tested periodically for changes in morphology and enzyme production and for evidence of C-type RNA virus production by assay for reverse transcriptase activity in tissue culture fluids¹³. The virus-specific nature of the enzyme was confirmed by inhibition

Table 1 Enzyme production by clonal lines of myeloid cells

Cell line	No. clones tested	No. clones positive (range of percent cells positive)			
		LAP	Esterase	Enzyme produced Myeloperoxidase	Lysozyme
Jones promyelocytic leukaemia	23	23 (85-91)	23 (90-95)	23 (91-95)	23 (88-95)
W/Fu myeloblastic leukaemia	15	0	0	0	15 (91-96)

Cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10% foetal calf serum (Colorado Serum Co.). The establishment of continuous tissue culture lines of the Jones chloroleukaemia and Wistar/Furth acute myelogenous leukaemia (W/Fu AML) have been reported¹². Multiple clonal lines derived from single cells of each leukaemia were selected by the microtitre plate technique according to published procedures¹⁹. Each was carried in tissue culture for 100 d. Histochemical assay methods for detection of myeloperoxidase, esterase and leukocyte alkaline phosphatase have been described¹⁰. A fluorescent antibody staining technique was used for detection of intracellular lysozyme. Cells were fixed and incubated with pre-absorbed rabbit serum, prepared against purified rat lysozyme, then washed and labelled with fluorescein-isothiocyanate conjugated, goat anti-rabbit globulin. Control preparations were incubated first with normal rabbit serum. Cells were scored using a $\times 1,000$ binocular microscope or fluorescent microscope by counting 1,000 cells in each of duplicate preparations for each clone.

with antibody to mouse C-type virus reverse transcriptase¹⁴. Several clones of the Jones chloroleukaemia were uniformly virus positive after 10 d in culture. The virus produced was shown to be C-type in origin by immunological tests for the interspecies antigenic determinant of the 30,000 molecular weight polypeptide of mammalian C-type virus¹⁵, and by electron microscopic analysis. It seemed likely that this was an endogenous rat virus. In spite of the continuous production of C-type virus for more than 300 d *in vitro*, there was no detectable change in promyelocyte morphology or in the strength of production of the four myeloid enzymes (data not shown). W/Fu AML cells remained virus-negative for several months in culture in spite of growth characteristics comparable with Jones chloroleukaemia cells. After 150 d *in vitro*, some W/Fu AML clones were found to be producing low levels of an indistinguishable rat C-type virus, yet there was no detectable change in myeloblast morphology or myeloid enzyme production.

Our results provide the first demonstration of the production of multiple lysosomal enzymes by clonal lines of myeloid cells and imply that all myeloid stem cells can produce multiple enzymes. The fact that only one myeloid enzyme was produced by W/Fu AML cells possessing a primitive myeloblast morphology, while several enzymes were synthesised by the more differentiated promyelocytic line, supports the finding in bone-marrow preparations of low or absent levels of myeloid enzymes in morphologically undifferentiated cells. If it is true that production of a single enzyme by a myeloblastic leukaemia results from maturation arrest rather than malignant transformation of a unipotential stem cell destined to produce only one enzyme, then stimulation of maturation of these myeloblasts should lead to production of other enzymes. These studies are in progress.

Both spontaneous¹⁶ and chemical^{17,18} activation of C-type viruses endogenous to murine cells in culture have been demonstrated previously. The present studies indicate that the spontaneous release of C-type virus by myelogenous leukaemias in culture occurred in the absence of any detectable morphological or biochemical alteration.

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Spontaneous lymphokine synthesis by human blood mononuclear cells

LYMPHOCYTES, after antigenic stimulation, may synthesise and release biologically active soluble factors other than antibodies. These mediators were termed lymphokines by Dumonde¹, and the most extensively studied and best characterised are migration inhibitory factors which can inhibit the migration of macrophages or leukocytes: this is the property used for their *in vitro* bioassay. Apart from antigens, various other stimuli may trigger lymphokine synthesis by lymphocytes, for example, polyclonal mitogens², anti-immunoglobulin or membrane Fc or C3-receptor reactions^{3,4}. Furthermore, migration inhibitory activity has been found in the long term culture supernatants of some established lymphoid and even non-lymphoid cell lines^{5,6}. We report here that human blood mononuclear cells can, apparently without any inducing agent, produce material inhibiting migration of homologous leukocytes.

Mononuclear cells from healthy human individuals were obtained by Ficoll-Isopaque centrifugation of heparinised peripheral blood⁷, or by incubating sedimented leukocytes for 30 min. at 37 °C with carbonyl iron and removing the phagocytic cells with a magnet. If not otherwise stated, 2×10^6 cells were cultured in 'universal' polystyrene containers (Sterilin, Richmond, Surrey) for 3 d in 1 ml of HEPES-buffered (10 mM) tissue culture medium 199 supplemented with 10% inactivated horse serum. Penicillin (70 IU ml⁻¹), streptomycin (70 µg ml⁻¹) and bicarbonate (0.1 M) were added to the medium, final pH 7.4. When puromycin was added to the control tubes its concentration was 5 µg ml⁻¹, which in previous tests was found to block the synthesis of migration inhibitory activity by cultured phytohaemagglutinin-stimulated cells. This concentration did not inhibit the effect of migration inhibitory activity on leukocytes and caused 10.7% inhibition of the migration of normal leukocytes in direct tests. The cell-free culture supernatants were tested for the presence of migration inhibitory activity with normal human blood leukocytes by the method of Clausen⁸. Each supernatant was tested in five or six replicate determinations. In our hands this method worked with standard deviations ranging from 2.2 to 11.8%, mean 6.6%.

In a series of experiments with mononuclear cells from 18 individuals, supernatants from unstimulated cultures produced 12.8% (mean) inhibition of migration when compared with migration areas of leukocytes in the corresponding medium. The phenomenon was not constant as significant inhibition (*t* test, paired samples) occurred in 11 out of 18 supernatants. In those 11 individuals the mean percentage of inhibition was 21.2 and the range was from 13.5 to 28.3%. That this was due to material actively synthesised by cultured cells is demonstrated in Table 1. The phenomenon did not occur if protein synthesis was blocked by cold or puromycin. The fact that, in the presence of puromycin, areas of migration were 9% smaller than in controls is explained by the direct effect of puromycin on leukocyte migration. Therefore, the use of puromycin controls in later experiments results in under- rather than overestimation of migration inhibitory activity.

Obviously, something in the conditions in which the cells remained during culture could stimulate the cells and bring about the synthesis of lymphokines. For example, foetal calf serum has been shown to be mitogenic for mouse B cells⁹. Therefore media supplemented with horse serum or autologous serum or no serum were compared for their possible stimulating activity (Fig. 1). The culture supernatants contained migration inhibitory activity regardless of the presence or nature of serum. Autologous serum did not affect inhibition. This indicates that

Table 1 Effect of blocking of protein synthesis on the generation of migration inhibitory activity in unstimulated culture supernatants

Cell donor	Medium control	37 °C	Supernatant of unstimulated cells cultured at 37 °C with puromycin	4 °C
1	25.2±1.9*	18.8±0.7	23.4±2.7	
2	36.5±3.1	29.1±0.7	29.3±0.9	
3	30.7±2.6	23.0±1.1	28.9±2.5	
4	30.3±1.4	23.9±0.6	29.9±2.2	
5	39.5±1.4	28.3±1.4	34.6±1.5	
6	48.0±5.7	40.6±4.4	45.6±4.4	
Mean	35.0	27.3	32.0	
		-----P<0.01-----		
7	21.8±0.8	18.1±0.8		20.8±0.4
8	23.9±1.1	20.6±0.5		27.0±2.3
9	21.8±0.8	15.2±0.8		19.0±1.0
10	23.9±1.1	18.6±1.3		25.8±0.5
11	16.3±1.1	13.9±1.6		15.0±1.2
Mean	21.5	17.3		21.5
		-----P<0.02-----		

* Area of leukocyte migration in mm². Mean ± s.d. of five or six replicate determinations.

antibodies were not responsible for the occurrence of this phenomenon.

It is possible that the cells were stimulated during isolation and preparation before culture. Heparin was an unlikely stimulant because the phenomenon was also noticed in cultures of cells originating from blood samples defibrinated using glass beads. Another possibility was Ficoll-Isopaque. To investigate this question we separated mononuclear cells by Ficoll-Isopaque centrifugation, by the carbonyl iron method and by a combination of both. The results (Fig. 2) suggest that the stimulation takes place even in cultures not exposed to Ficoll-Isopaque.

The nature of the cells responding in those conditions was investigated by separating mononuclear cells by the sheep red

Fig. 1 The role of serum as a possible cell stimulating agent. The cells were cultured with (○) or without (●) puromycin in the media indicated and the supernatants were tested with leukocytes for migration inhibitory activity. Medium control: leukocyte migration in the corresponding medium supplemented with horse serum, that is, not culture supernatant. -----, Individual experiments; ———, means. *a*, Medium control; *b*, horse serum; *c*, autologous serum; *d*, no serum.

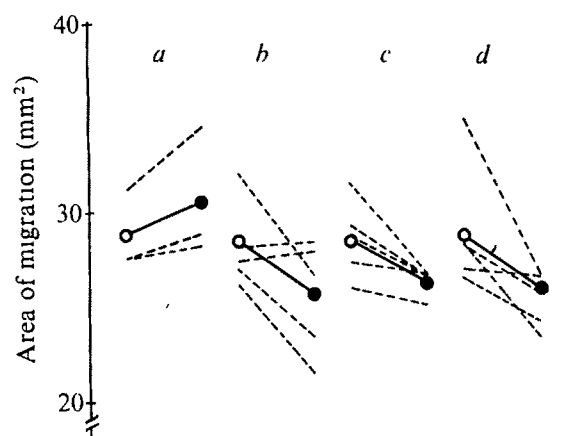
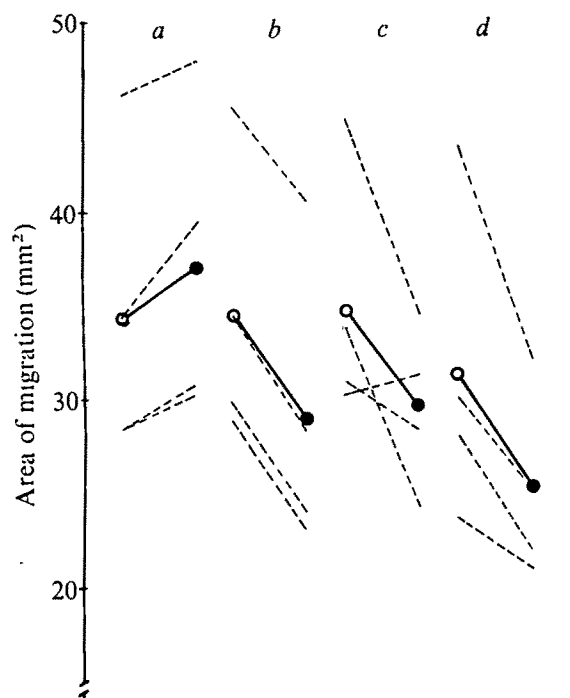


Fig. 2 Effect of the technique of isolation of mononuclear cells on the generation of migration inhibitory activity by unstimulated cells. For explanations, see legend to Fig. 1. *a*, Medium control; *b*, carbonyl iron; *c*, Ficoll-Isopaque + carbonyl iron; *d*, Ficoll-Isopaque.

blood cell-rosette technique¹⁰ into T cell-rich and B cell-rich populations. Preliminary experiments suggest that migration inhibitory activity is absent in T cell-rich cell supernatants and the responding cells are B cells and/or monocytes.

We have shown that this lymphokine-like activity in the supernatants of unstimulated cell cultures is brought about by an active synthetic process of cells and that its biological effect is indistinguishable from that of the migration inhibitory lymphokine. We have, however, no chromatographic data to support the conclusion that the phenomenon is caused by a definite migration inhibitory lymphokine. The synthesis of lymphokine(s) by apparently unstimulated human mononuclear cells in conventional culture conditions could be explained in several ways. First, the cells could have been stimulated already *in vivo* although, in our opinion, this is unlikely. Second, in theory, any of the steps in the isolation and preparation of cells could provide the necessary stimulus for the activation of cells. For example, guinea pig spleen B cells, after isolation on a nylon wool column can produce a chemotactic lymphokine without further stimulation⁴. Our experiments have failed so far to prove that any of the substances or procedures we used could act as a cell activating stimulus. Third, these cells may be activated during culture by the surface of the culture vessel on which they settle.

It has become popular to separate B and T lymphocytes and investigate their responses to mitogens, antigens or other

stimuli. In view of the various mechanisms of cooperation or amplification in the response of lymphocytes to these agents, our findings of mediator synthesis by a proportion of the mononuclear cells without evident stimulus should warrant careful interpretation of the claims of B or T cell specificity of various stimuli.

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Anti-C3 antibodies in neonatal saliva

ANTIBODIES which react with bound complement components, in particular the third component C3, have been found in the parotid saliva of most normal subjects¹. These antibodies have several unusual properties². Their binding to complement is dependent on calcium ions, they can be inhibited by small amounts of native C3 and although they belong to the IgA class they are macromolecules with a sedimentation coefficient greater than 19S. The titres in mixed saliva are substantially lower than in parotid saliva¹ and the reduction in titre results from the presence of C3 in mixed saliva that combines with the antibody and neutralises its activity. While investigating the possibility that crevicular fluid was the source of C3 in the mouth, we examined samples of saliva from newborn babies. High antibody titres were found in these samples. We report here on the prevalence and properties of this antibody in neonatal saliva.

Whole saliva was collected from 36 babies, 21 male and 15 female, between 30 min and 4 h after birth and before the first milk feed. The saliva sample was collected by means of a small sterile sponge placed in the mouth between the gum and the cheek. The sponge was removed after a few minutes, weighed and mixed with a known volume of saline. After centrifugation the supernatant was separated and stored at -20°C . Antibody activity to complement was determined by a direct agglutination technique using sheep red blood cells coated with human serum¹. High agglutinating titres were found in all 36 samples, the mean titre being 1:3,000 (range 1:500–1:8,000). Agglutination of the cells was calcium dependent and could be reversed by addition of EDTA (final concentration 0.01 M).

The properties of this antibody were examined using saliva samples from six babies. Similar properties were found in all samples and these are summarised in Table 1. Fractionation of saliva on Sephadex G200 revealed that the activity was localised to the void volume fractions, indicating that the molecular weight of this antibody was greater than 10^6 .

Agglutination of complement-coated cells by antibody could be inhibited by small amounts of serum, by purified C3 and by a mixture of the C3 products, C3c and C3d. The inhibitory activity of the C3c and C3d mixture remained unaltered after heating at 56°C for 30 min (conditions which abolish the antigenicity of the C3d fragment)³. Some activity against the C3d fragment could be demonstrated by using the red cells obtained from a patient with cold agglutinin disease; these cells were agglutinated at low titre (1:2–1:8) by half of the neonatal saliva samples tested.

Table 1 Properties of anti-C3 antibodies in neonatal saliva

Inhibition by 0.01 M EDTA	+++
Inhibition by whole serum	+++
Reaction with zymozan	—
Sensitivity to trypsin	+
Inhibition by N-acetyl-D-glucosamine	—
Inhibition by mercaptoethanol	+++

+++ , Strong; + , weak; — , absent.

The concentration of IgA in saliva begins to rise a few days after birth^{4,5}. Very little information is available, however, on the concentration of immunoglobulin in saliva collected within a few hours of birth. The concentration and class distribution of immunoglobulin in neonatal saliva was, therefore, determined in 14 samples using a modified radial immunodiffusion assay. This was performed as described previously⁶ except that mono-specific anti-immunoglobulin sera were incorporated into 0.8% 1-mm thick agar plates at a dilution of 1 in 80 and the samples were placed in 3-mm wells and allowed to react for 48 h at 37°C . The lowest detectable concentrations by this method were $2\text{ }\mu\text{g ml}^{-1}$ for IgG and IgM and $1\text{ }\mu\text{g ml}^{-1}$ for IgA. IgM and IgG were present in all unconcentrated samples of neonatal saliva. IgA, however, was detectable in only five of the 14 samples. The mean values and concentration range of the three immunoglobulins in saliva are summarised in Table 2. The distribution of the anti-C3 antibody within the immunoglobulin classes was determined using purified antibody. Pooled neonatal saliva (20 ml) was incubated with complement-coated red cells and after washing, the antibody was eluted from the cell surface by 0.01 M EDTA. The eluate was further concentrated tenfold by ultrafiltration and the concentration of immunoglobulin was determined by radial immunodiffusion. IgA was the only immunoglobulin detectable in the concentrated eluate.

The antibody titres found in neonatal saliva are three to five times higher than the titres found in the parotid saliva of normal adults. The molecular weight, immunoglobulin class and properties of both of these antibodies, however, seem to be identical. Both antibodies are also readily inhibited by native C3, a finding which contrasts markedly with the specificity shown by the serum antibodies which react with fixed complement. Serum immunoglobulins react with the hidden determinants in the complement molecule that are exposed on fixation and they are only weakly inhibited by native C3, whereas salivary antibodies react with the antigens present on native C3. It has been suggested that the anti-C3 antibodies in saliva arise following immunisation, possibly with bacteria which cross react with native C3 (ref. 7). The presence of very high titres in neonatal saliva within a few hours of birth, at a time when bacterial colonisation of the mouth is only beginning to take place⁸, suggests, however, that these antibodies are not produced by the foetus in response to immunisation with oral bacteria. The finding of this antibody in amniotic fluid at 34 weeks supports this view (unpublished results).

Table 2 Concentration of IgG, IgA and IgM in neonatal saliva

	IgG	IgM	IgA
Number positive	14/14	8/8	5/14
Mean ($\mu\text{g ml}^{-1}$) \pm s.e.	14.2 ± 4.5	4.75 ± 1.2	$3.8 \pm 2.0^*$
Range	2–64	2–10	1–11

* Mean of five positive samples.

Attention has already been drawn to the possible relevance of these antibodies to the understanding of the nature of immunological tolerance⁷. Their occurrence in saliva implies that a population of B cells persists during foetal development and continues to survive into neonatal and adult life. The reasons for the persistence of the cells in salivary tissue are not

known but may be related to local factors or possibly to their requirement for calcium ions before binding to C3 occurs.

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Antisera to human B-lymphocyte membrane glycoproteins block stimulation in mixed lymphocyte culture

HUMAN B cells are frequently differentiated from T cells by the ready demonstration of immunoglobulin (Ig) on their surfaces¹. In the mixed lymphocyte reaction (MLR) the responding cells are mainly T cells²⁻⁴, whereas B cells have been suggested to be the primary stimulating cells⁵. The effect on the MLR of a specific anti-B-cell serum is therefore of considerable interest. Here we describe rabbit antisera to membrane glycoproteins of cultured human B cell lines which react only with Ig-bearing peripheral blood lymphocytes and which completely block the MLR *in vitro*.

During attempts to make xenoantisera to HL-A antigens, rabbits were immunised with papain-solubilised HL-A antigens purified from the human B cell line RPMI 4265 by previously reported techniques^{6,7}. Subsequently, the antisera were examined to determine the nature of the membrane molecules with which they reacted. The method of analysis involved incubating these antisera with detergent-solubilised membranes⁷ from cultured human lymphoblastoid lines (labelled with radioactive amino acids^{8,9}), precipitating the immune complexes with goat anti-rabbit IgG, and subjecting the washed precipitates to acrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS)⁸. When tested against solubilised membranes derived from B cell lines (in this case, Ig-secreting lines), two of the sera reacted with molecules with apparent molecular weights of 35,000 and 27,000, in addition to what we presumed were HL-A glycoproteins (43,000) and β_2 -microglobulin (11,000)^{7,9-12}. When the sera was tested with labelled material from a human cell line (HSB) with T cell characteristics^{13,14}, however, only 43,000 and 11,000 molecular weight peaks were evident. Figure 1a shows profiles obtained on SDS-gel electrophoresis of a mixture of two immune precipitates. One was formed by incubating one of the sera (serum C) with ¹⁴C-amino acid-labelled, detergent-solubilised membranes from HSB, and the other by incubating serum C with ³H-amino acid-labelled membranes identically solubilised from the cell line SB. SB is an Ig-secreting cell line derived from the same donor as HSB^{13,14}. The additional molecules detected in the SB preparation can be seen clearly. The species with molecular weights 35,000 and 27,000 can also be precipitated from soluble membranes of B cell lines labelled with ³H-glucosamine, under conditions where β_2 -microglobulin (known not to contain carbohydrate¹⁵) is not labelled^{6,7}. This suggests that the molecules on B cell lines are glycoproteins.

In an attempt to make a specific reagent against these

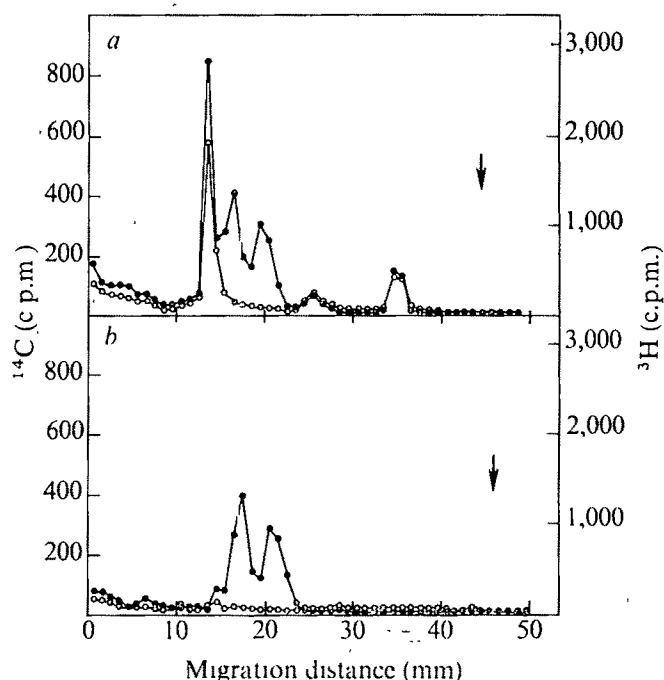


Fig. 1 SDS-gel electrophoresis profiles of immune precipitates of ¹⁴C-amino acid-labelled HSB membranes solubilised in 0.1% Nonidet P-40 (NP-40), 0.15 M NaCl, 0.01 M Tris, pH 8.0 (○), and ³H-amino acid-labelled SB membranes similarly solubilised (●). *a*, A mixture of precipitates formed with each preparation and serum C unabsorbed, *b*, a mixture of precipitates formed with each preparation and serum C absorbed three times (30 min, 25 °C) with 25×10^6 HSB cells per 100 μ l of serum. Insoluble material was removed from detergent-treated membranes¹⁻⁷ by centrifugation at 100,000g for 90 min. An aliquot (25 μ l) of solubilised membranes (200,000 c.p.m.) was incubated with 1.5 μ l of serum for 1 h at 25 °C, and an excess of goat anti-rabbit IgG (adjusted to 0.1% NP-40) was added to effect precipitation. After 30 min at 37 °C, and a minimum of 1 h at 4 °C, the precipitates were washed three times with 0.1% NP-40 in 0.15 M NaCl, 0.01 M Tris, pH 8.0, twice with water, and subjected to electrophoresis on 13% SDS gels prepared according to Laemmli⁸. The gels were cut into 1-mm slices and counted as previously described^{6,7}. Calibration proteins used in molecular weight estimations were bovine serum albumin (67,000), ovalbumin (45,000), chymotrypsinogen A (25,000) and cytochrome *c* (12,000). The arrows indicate the position of the bromophenol blue marker dye.

glycoproteins, serum C was absorbed extensively with the T cell line, HSB. After absorption the serum failed to precipitate the 43,000 and 11,000 molecular weight peaks from the labelled membrane preparations (Fig. 1b). The reaction of the absorbed serum (serum C_{abs}) with the additional glycoprotein peaks in the SB preparation remained intact.

Serum C_{abs} was also examined for complement-mediated cytotoxicity of peripheral blood lymphocytes (PBL), using the ⁵¹Cr-release assay¹⁶. The results are shown in Fig. 2. Before absorption, serum C was highly lytic for human lymphocytes, giving 80% ⁵¹Cr-release, with a 50% release titre of 1 in 330. After absorption with HSB, approximately 25% ⁵¹Cr-release was observed (with a background release of 10%) to an antiserum dilution of 1 in 200 to 1 in 300. This experiment strongly suggests that the serum C_{abs} is cytotoxic for a minor population of peripheral lymphocytes.

The B cell specificity of the serum was demonstrated by two kinds of immunofluorescence study. In the first experiment, aliquots of PBL were incubated at 0 °C with serum C_{abs}, with a rabbit antiserum to human immunoglobulins (rabbit anti-human Ig, Nutritional Biochemicals), or with a mixture of the two sera. In all three cases both sera were diluted 1 in 5 in phosphate-buffered saline (PBS) containing 5% foetal calf serum with dinitrophenol added to 2.5 mM to prevent capping. Fluorescent cells were counted (at least 50 positive cells per sample) after washing and staining with a goat antiserum

to rabbit immunoglobulins conjugated with fluorescein isothiocyanate (FITC-goat anti-rabbit Ig, Meloy Laboratories). PBL treated with serum C_{abs} gave 18.5% positive fluorescence, those treated with rabbit anti-human Ig gave 16.6% positive fluorescence, and cells treated with a mixture gave 17.7% positive fluorescence. No summation was observed with the antiserum mixture, suggesting that both were reacting with the same population. Cells treated with a pre-immune bleed of serum C followed by FITC-goat anti-rabbit Ig showed only 1.3% positive fluorescence.

In a second experiment, designed to determine whether Ig-positive cells were lysed by serum C_{abs}, 10⁷ PBL were incubated with 1 ml of the serum (diluted 1 in 30 in PBS, 30 min, 37 °C), centrifuged (1,000g, 5 min), resuspended in and incubated in 1 ml of rabbit complement (30 min, 37 °C). The cells were then applied to a discontinuous Ficoll-Hypaque gradient¹⁷ and centrifuged (2,000g, 30 min) to remove dead cells. The cells isolated from the interface (greater than 95% viable by Trypan blue staining) were washed and incubated with rabbit anti-human Ig or normal rabbit serum, followed by FITC-goat anti-rabbit Ig as described above. Lysed cells showed 1.7% positive fluorescence with normal rabbit serum and 2.5% positive fluorescence with rabbit anti-human Ig. Cells treated identically, but with normal rabbit serum and complement, showed 2.4% positive fluorescence with normal rabbit serum and 13.4% positive fluorescence with rabbit anti-human Ig. This experiment clearly shows that most (if not all) of the minor population of PBL lysed by serum C_{abs} are Ig-positive, that is, B cells.

Rabbit antisera to human β_2 -microglobulin (β_2m) have been reported to block the MLR in humans¹⁸, as have alloantisera to HL-A antigens^{19,20}. We have been conducting MLR blocking experiments with a rabbit antiserum to β_2m (ref. 7) and another to purified HL-A antigens solubilised by papain (serum A). These antisera react only with the 11,000 and 43,000 molecular weight HL-A-related species when assayed on B cell line labelled membranes by immune precipitation and SDS-gel electrophoresis as described in Fig. 2. These sera and serum C_{abs} were assayed for their ability to block the MLR. Figure 3 shows the results of these experiments. Clearly serum C_{abs} is the most effective at blocking the MLR. The response is reduced to background levels even at a dilution of 1 in 16,000 and is still approximately 50% inhibited at a dilution of 1 in 10⁶. Taking

Fig. 2 Lysis of peripheral blood lymphocytes by serum C (●), and serum C absorbed as in Fig. 1 with the T cell line HSB (○). Dilutions of the sera (10 μ l) were incubated (30 min, 37 °C) with 10 μ l of dextrose-gelatin veronal buffered saline (Grand Island Biological) containing 2 \times 10⁴ PBL (prepared by the method of Boyum¹¹) labelled with Na⁵¹CrO₄ (ref. 16). Rabbit complement (10 μ l) was added and the cells incubated for a further 30 min at 37 °C. After addition of 50 μ l of PBS containing EDTA (15 mM) and centrifugation, aliquots of the supernatants were counted. One hundred per cent release is the total c.p.m. incorporated into 2 \times 10⁴ cells, and the dashed line indicates the ⁵¹Cr-release (10%) when no antiserum was added.

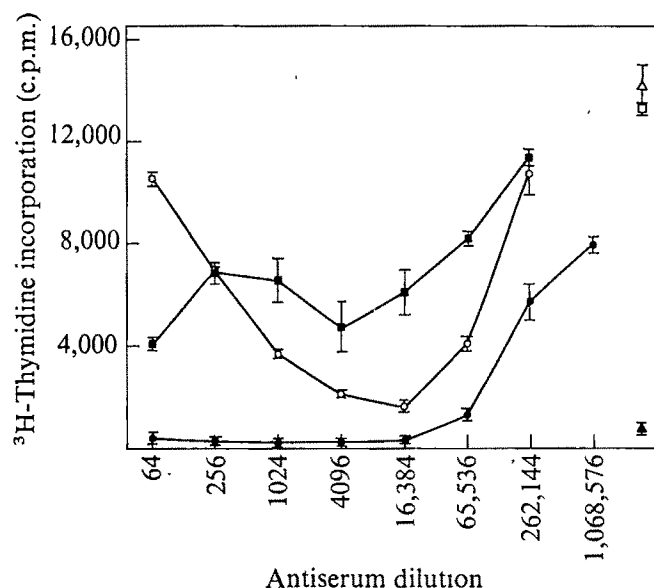
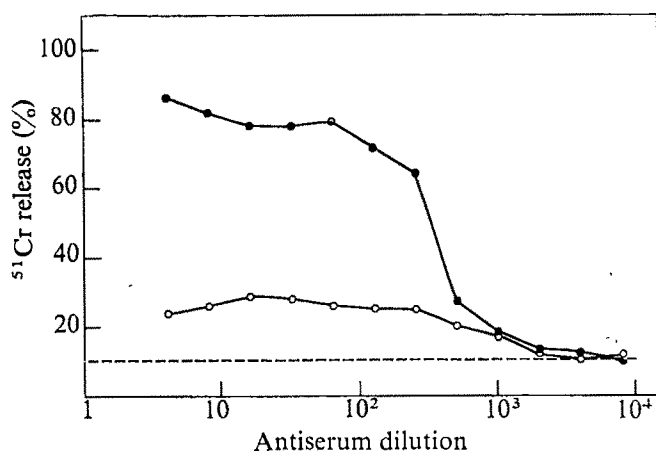


Fig. 3 Effect of various sera on the mixed lymphocyte response: serum C absorbed with HSB (●); serum A, a rabbit anti-HL-A (papain-solubilised) serum (○); rabbit anti- β_2 -microglobulin (■). Controls indicated are: responder cells plus stimulator cells in the absence of rabbit serum (□); responder cells alone (▲); responder cells plus stimulator cells with serum C pre-immune bleed at a dilution of 1 in 64 (△). Vertical bars indicate the standard error of the mean. Stimulator cells were treated with mitomycin C (40 μ g/10⁷ PBL ml⁻¹) for 30 min at 37 °C. One way mixed lymphocyte cultures were set up in quadruplicate in U-well plates (Cooke Engineering). Responder cells (10⁵ per well) and stimulator cells (10⁶ per well) were incubated in 0.2 ml RPMI 1640, with 20% human type A serum and penicillin and kanamycin, containing the indicated dilutions of rabbit sera. All sera were heat-inactivated at 56 °C for 45 min. Cultures were collected using a Skatron cell harvester after 6 d at 37 °C in 5% CO₂ in air. ³H-Thymidine (1 μ Ci) was added per well 16 h before collection.

50% inhibition of the incorporation of ³H-thymidine as an endpoint, serum C_{abs} is one to two orders of magnitude more effective in blocking the MLR than rabbit anti-HL-A and anti- β_2m . The unusual shape of the curves for rabbit anti-HL-A and rabbit anti- β_2m seems to result from direct stimulation of the responder cells by the antisera. Controls with only responder cells and serum A or anti- β_2m added (not shown) gave increased ³H-thymidine incorporation at high concentrations of the sera. In similar controls no stimulation was observed by serum C_{abs}.

The fact that a serum which seems to be specific for B cells can block MLR is interesting, and may indicate that B cells are the predominant stimulator cells, as previously suggested⁴. In additional experiments (unpublished) we have shown that pretreatment of mitomycin C-treated stimulator cells with the anti-B-cell serum, followed by washing, blocks the MLR. Pretreatment of responder cells does not. We do not yet know, however, whether the serum reacts with other cells possibly involved in the MLR, such as macrophages, nor can we positively state that the antigens to which serum C_{abs} are directed are totally absent from T cells. They may be only operationally undetectable.

The nature of the papain-derived product which elicited the production of the B-cell-specific antibodies remains to be determined. At least four bands of apparent molecular weights between 20,000 and 32,000 appear on overloaded SDS gels of the antigen preparation used to produce serum C, in addition to the strong 34,000 and 11,000 molecular weight bands usually observed in papain-solubilised HL-A preparations^{5,6}. One or more of these species may be a papain cleavage product of the 35,000 or 27,000 molecular weight molecules. Experiments to characterise the immunogen(s) further are in progress. The possible relationship of the B cell glycoproteins to mouse Ia antigens, which are of similar molecular size²¹ and found

predominantly on B cells^{22,23} (though some are present on T cells²⁴) is also an area to be investigated. Other anti-human B-cell antisera have been reported^{25,26}, but the nature of the target antigens in these cases is unknown. A lytic antiserum specific for B cells with known target antigens promises to be a valuable reagent in human immunological studies.

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Receptor-mediated inactivation of early B lymphocytes

THE interaction of surface immunoglobulin (sIg), the antigen receptor of B lymphocytes, with ligands—whether antigen or anti-Ig antibodies—initiates a series of surface and cytoplasmic events which, depending on the nature of the ligand and the presence or absence of cooperative signals from other cells, has various outcomes (reviewed in ref. 1). Immediately on the binding of a polyvalent ligand to sIg, the complexes redistribute on the cell surface, a contractile event occurs, the complexes are endocytosed and shed from the membrane and eventually new receptors appear on the cell surface¹. This *in vitro* cycle, in the absence of cooperative interactions, does not lead to B cell differentiation into secreting plasma cells, whereas it does so when induced in the proper conditions involving helper cells^{1,2}.

A crucial step in this ligand-induced cycle is the re-expression of new receptors on the cell surface, a process involving protein synthesis and requiring the activity of microtubules¹. It is expected that an interruption at this step, leading to a lack of receptors on the cell surface, would be tantamount to an inactive B cell unable to respond to antigen. We report here the failure of B cells from young mice to re-express sIg after relatively brief exposure to anti-Ig antibodies. This failure denotes a peculiar sensitivity of early B cells to ligand-receptor interaction in the absence of cooperative effects and could readily explain several of the phenomena of unresponsiveness

seen during the neonatal period. It should be noted that B cells from young mice (until about 2 weeks of age) have characteristics different from most adult B cells in that they lack C3 receptors^{3,4}, cap poorly⁴ and bind more labelled anti-Ig antibodies⁴.

The basic experiments consisted of exposing spleen lymphocytes from young (up to 2 weeks after birth) or adult (8-16 weeks) mice to rabbit anti-mouse Ig antibodies (RAMG) for 1 or 24 h, after which the cells were cultured for several days in the absence of RAMG. Surface Ig was detected by immunofluorescence. When RAMG bound to an adult B lymphocyte, sIg-anti-Ig complexes were eliminated within minutes, leaving the cell membrane cleared of sIg. Then, within a few hours of culture in the absence of anti-Ig, the cell restored its sIg, completing the process by 8-24 h later. The B lymphocyte from a young mouse spleen however, did not behave in this way. Depending on the time the cell was exposed to RAMG, the early B lymphocyte restored its sIg poorly or not at all. As Fig. 1 shows, within 1 d of a 1-h or a 24-h RAMG incubation (both of which completely cleared the cell of sIg), most of the original adult B lymphocytes restored their sIg. In contrast, only about half of the early B lymphocytes re-expressed sIg after a 1-h RAMG treatment, and almost none re-expressed it after 24 h of treatment with RAMG.

Since about half of the early B lymphocytes restored their sIg after a single cycle of clearance, it seems unlikely that the sIg initially on the cell surface represented cytoplasmic Ig. To strengthen this conclusion and to test whether early B lymphocytes can ever completely regenerate their sIg, cells of both ages were treated with the proteolytic enzyme Pronase at doses known to eliminate surface Ig, and then observed for the re-expression of sIg. Figure 2 shows that both early and adult B lymphocytes completely regenerated their sIg after with Pronase clearance. Note also for the early cells that a portion treated with Pronase after 24 h of incubation with RAMG did not re-express sIg. This shows that Pronase did not represent some stimulatory activity which might reverse the

Fig. 1 Re-expression of sIg after treatment with RAMG (the RAMG used was a poly-specific rabbit anti-mouse Ig antibody previously shown to bind only to B lymphocytes in the mouse spleen). Young (6-9 d, *a*) or adult (2-4 months, *b*) C57BL/6 mouse spleens were teased into Hanks' balanced salt solution (HBSS) plus 1% HEPES plus 5% foetal calf serum and washed three times. They were then exposed at a concentration of 10^7 cells per ml to Mishell-Dutton medium¹⁵ alone or containing RAMG ($200 \mu\text{g ml}^{-1}$) for 1 h or 24 h at 37°C in a 5% CO_2 atmosphere. The dose of RAMG was one that totally cleared the cells of sIg. After the RAMG treatments, cells were washed five times, resuspended at 10^7 cells per ml in fresh medium and cultured for various times. For immunofluorescence the cells were washed once, pelleted, resuspended in $50 \mu\text{l}$ of medium and $50 \mu\text{l}$ of fluoresceinated RAMG (FITC-RAMG) ($500 \mu\text{g ml}^{-1}$, F.P. ratio approximately 6:3). The cells were incubated for 30 min at 4°C , washed twice, resuspended 10^7 per ml, and live cells were scored in a Lertz fluorescence microscope. —, Control; ---, 1-h RAMG; - - -, 24-h RAMG

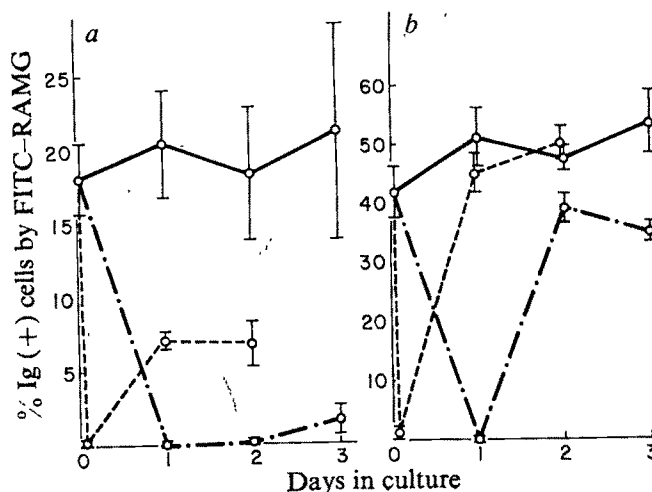


Table 1 Effect of RAMG treatment on LPS mitogenesis

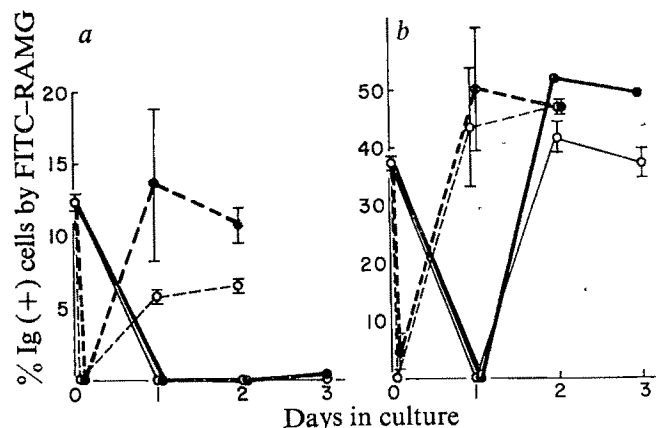
Incubation (h)	Incubation medium	LPS in culture	Experiment 165			Experiment 167			Experiment 169			Averages		
			c.p.m. 10 ⁶ cells	E-C	E/C	c.p.m. 10 ⁶ cells	E-C	E/C	c.p.m. 10 ⁶ cells	E-C	E/C	E-C	E/C	
Young cells														
1	Medium	—	5,319± 97			7,605± 276			4,842± 407					
1	Medium	+	20,662±1,047	15,343	3.88	46,455±1,648	38,850	6.0	52,601± 808	47,759	10.86	33,964	6.95	
1	RAMG	—	4,854± 138			6,770± 381			3,973± 338					
1	RAMG	+	5,787± 671	933	1.19	17,294± 271	10,524	2.55	17,406± 813	13,433	4.38	8,297	2.71	
24	Medium	—	3,016± 362			5,339± 122			3,589± 189					
24	Medium	+	10,739± 278	7,724	3.56	15,085± 245	9,746	2.83	18,184± 696	14,595	5.07	10,688	3.82	
24	RAMG	—	3,848± 157			7,393± 314			4,910± 189					
24	RAMG	+	2,083± 146	-1,765	0.54	4,456± 195	-2,937	.60	3,660± 225	-250	.94	-1,650	.69	
Adult cells														
1	Medium	—	10,009± 654			10,979± 430			7,447± 220					
1	Medium	+	71,939±2,033	61,930	7.19	77,002±1,393	66,023	7.01	74,690±1,180	67,243	10.03	66,065	8.08	
1	RAMG	—	3,092± 288			3,792± 155			5,016± 56					
1	RAMG	+	35,960± 268	32,868	11.63	39,629± 561	35,847	10.45	62,899±2,185	57,883	12.54	42,199	11.54	
24	Medium	—	4,582± 117			6,972± 375			4,540± 300					
24	Medium	+	60,305± 412	55,723	13.16	59,311± 321	52,339	8.51	62,367±1,027	57,827	13.74	55,296	11.80	
24	RAMG	—	3,362± 692			2,180± 54			1,909± 68					
24	RAMG	+	61,059±3,344	57,697	18.16	37,966±1,660	35,786	17.42	55,392±1,448	53,483	29.02	48,989	21.53	

Young and adult C57BL/6 cells were incubated for 1 or 24 h in Mishell-Dutton medium containing RAMG (200 µg ml⁻¹) or no RAMG. They were then washed five times in HBSS plus HEPES plus FCS and resuspended to 10⁶ per ml in RPMI-1640 with penicillin and streptomycin and 5% FCS and 2 mM L-glutamine alone or with LPS (10 µg ml⁻¹). Each point was done in triplicate. After 24 h of culture in 5% CO₂, each tube was pulsed with 1 µCi in 50 µl of ³H-thymidine (2 Ci mmol⁻¹). The incorporation of the labelled thymidine into trichloroacetic acid-insoluble material was determined 24 h later. The results are expressed as the arithmetic mean of triplicate cultures ± s.e.m. E-C, Experimental mean—control mean; E/C, experimental mean/control mean.

effects of the 24-h treatment with RAMG. The main conclusion from the Pronase experiments is, therefore, that early B lymphocytes can re-express their sIg as completely and as rapidly as adult B lymphocytes but are specifically inactivated by interaction with RAMG.

The next question asked was whether these early B lymphocytes were inactivated or totally deleted from the cultures (that is, killed). To determine this, the spleen cells were examined after exposures to RAMG for the presence of another⁵,

Fig. 2 Re-expression of surface Ig after treatment with Pronase. Young (6–8 d, *a*) and adult (2–4 months, *b*) C57BL/6 cells were treated with Pronase (2 mg ml⁻¹) (*Streptococcus griseus* protease) and DNase II (20 µg ml⁻¹) for 1 h at 37 °C in HBSS plus HEPES, washed five times and then cultured and assayed as in Fig. 1. Treatment with Pronase was either immediately after cell collection or after 24 h culture in Mishell-Dutton medium containing RAMG (200 µg ml⁻¹) and three washes. For comparison, re-expression after incubations of 1 and 24 h in Mishell-Dutton medium containing RAMG (200 µg per ml) are also shown. ○—○, 1 h RAMG; ○—○, 24 h RAMG; ●—●, 1 h Pronase; ●—●, 24 h RAMG, 1 h Pronase



independent⁶ B lymphocyte marker, the I region-associated antigens (Ia). As all panels of Fig. 3 show, the surface distribution of Ia was unaffected immediately after the clearance of sIg by RAMG in early and adult B lymphocytes. One day after incubation with RAMG, adult B lymphocytes still showed their Ia and had restored their sIg. In contrast, early B lymphocytes also retained their Ia but had restored their sIg poorly or not at all. We concluded, therefore, that these early B cells were still present in the culture and alive at this time but had failed to re-express their sIg. Two days after the end of a 24-h treatment with RAMG the situation seemed to be changing, however. The percentage of early Ia-positive cells was declining, implying that, as a result of the prolonged ligand-receptor interaction, the original early B lymphocytes were either dying or were changing other cellular characteristics.

In addition to surface markers, the functional property of lipopolysaccharide (LPS)-induced mitogenesis was also studied after receptor clearance. Table 1 shows that the LPS mitogenic response of adult B lymphocytes was affected minimally, if at all, after treatment with RAMG. In striking contrast, early B lymphocytes had a marked reduction in LPS-induced mitogenesis after the same procedures. Thus, the failure to re-express receptors after RAMG clearance was accompanied by the loss of responsiveness to the potent B cell mitogen LPS.

Although the mechanism of this receptor-mediated inactivation of early B lymphocytes is not clear, certain conclusions can be drawn.

First, the inactivation seems to be a direct consequence of the interaction of RAMG with sIg and does not involve suppressor-type T-cell effects. In this system the RAMG only interacts with B lymphocytes. Furthermore, in experiments involving mixtures of adult and young spleen cells, each population reacts independently, neither one influencing the other in any direction. Second, this inactivation was not associated with proliferation of B cells after treatment with RAMG. We have failed repeatedly to detect any mitogenic

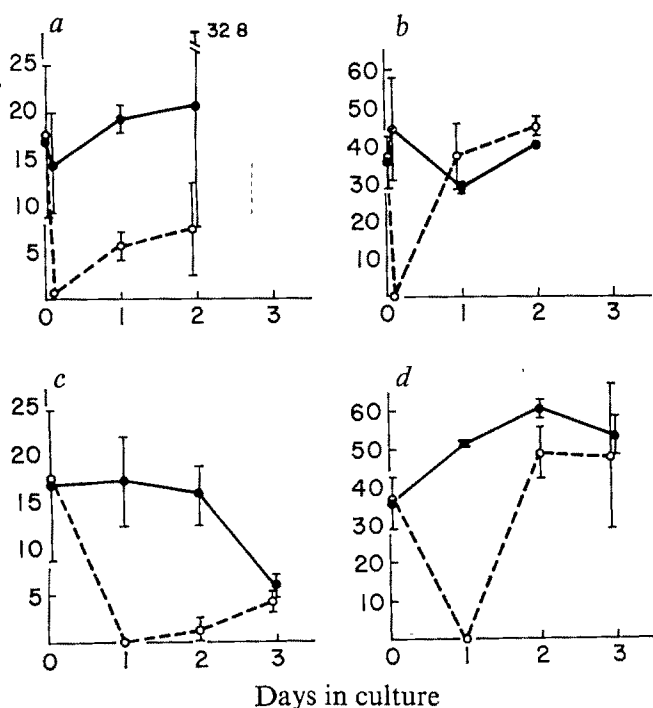


Fig. 3 Re-expression of surface Ig in relation to surface Ia. The four panels show young and adult AKR cells treated for 1 and 24 h with RAMG as in Fig. 1. For each assay point, a tube of cultured cells was washed once, pelleted, resuspended in 50 μ l rhodamine-conjugated RAMG (R-RAMG), (1.3 mg ml⁻¹), incubated for 30 min at 4 °C, washed three times, and resuspended in 20 μ l normal mouse serum. Twenty microlitres fluorescein-conjugated ATH anti-ATL spleen cell serum (F anti-Ia) were added, and the cells were incubated for 30 min at 4 °C, washed twice and resuspended to 10⁷ per ml. The cells were then scored for the presence of Ig (rhodamine stain) and Ia (fluorescein stain). *a*, Young cells, 1 h RAMG; *b*, adult cells, 1 h RAMG; *c*, young cells, 24 h RAMG; *d*, adult cells, 24 h RAMG. ●, % cells Ia (+) by FITC-a Ia; ○, % cells Ig (+) by R-RAMG.

effect of RAMG on early or adult murine B lymphocytes. Finally, this receptor-mediated inactivation seems to be controlled by some intracellular mechanism independent of the presence or absence of ligand-receptor complexes on the membrane. Ault *et al.*⁷ showed that after binding a highly tolerogenic polypeptide of *D*-amino acids, primed adult mouse spleen cells did not regain antigen-binding capability. Also, the complexes were not totally cleared from the cell surface. One could explain these results⁷ by postulating a relationship between clearance of complexes and expression of new receptors. This explanation does not apply to our experiments, however, since the early B cell does clear its surface of complexes. Also, even if some long lasting complexes were present, the Pronase should have removed them and released the cell.

These results show that the interaction of the antigen receptors of an early B lymphocyte with a specific ligand leads to the inactivation of two fundamental B cell processes—receptor re-expression and LPS-induced mitogenesis. This phenomenon may be the basis for several situations of immunological unresponsiveness. The inactivation reported here could explain, in part, the phenomenon of allotype suppression, as well as the analogous cases of suppression of specifically committed cells by anti-receptor antibody^{8,9} and of cells bearing a particular Ig class by class-specific antibody¹⁰. Further, young animals are notoriously easy to render specifically tolerant towards antigen. Although other cells and mechanisms are probably also operative¹¹, a specific antigen may directly turn off the early B lymphocytes by the process discussed here. Studies are in progress evaluating this point directly on antigen-binding lymphocytes. Finally, the inactivation of early type B lymphocytes could also have a role in adult tolerance. There is

evidence that adult mouse spleen⁴ and bone marrow¹² contain some B lymphocytes of the type constituting the young spleen. These may be precursors which constantly repopulate the pool of competent adult B lymphocytes¹³. It is possible that the state of unresponsiveness in the adult, although complex, could be prolonged by the ligand-induced inactivation described here, having as its target the early type of B cell found in bone marrow. In this respect, it has been shown that adult bone marrow lymphocytes are very easily rendered tolerant by antigen *in vitro*¹⁴.

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In vitro* recognition of carcinogen-induced local denaturation sites in native DNA by S₁ endonuclease from *Aspergillus oryzae

It is known^{1–4} that the AAF-addition products obtained in the reaction of the ultimate carcinogen, N-acetoxy-N-2-acetylaminofluorene (AAAF) with DNA *in vitro* are essentially the same as those isolated from liver DNA of rats fed with N-2-acetylaminofluorene (AAF). Thus, the modifications of DNA secondary structure observed *in vitro* should reflect DNA alterations arising *in vivo*. It has been shown^{5–9} that the main alteration induced by the fluorene ring is the creation of locally disorganised regions inside the double-helical structure. We now show that S₁ endonuclease (EC3.1.4.) from *Aspergillus oryzae* is able to excise the local regions of denaturation induced in native DNA molecules by AAAF.

When the kinetics of the hydrolysis of a carcinogen-modified native DNA sample (DNA-AAF) by endonuclease were measured they were found to be intermediate between the kinetic curves of native and fully denatured DNA (Fig. 1a). Moreover, by following A₃₀₅, the acid-soluble material was shown to contain fluorene (Fig. 1b). Analogous measurements using native DNA modified by two derivatives of AAF are also reported.

The first, N-acetoxy-N-2-acetylaminofluorene (AAAF), is a model of the ultimate metabolite of the strong carcinogen, N-2-acetylaminofluorene (AAF)¹⁰. According to the general mechanism of activation of arylamides¹, the second, N-acetoxy-N-2-acetylaminofluorene (AAAF), may be a metabolite of the non-carcinogen N-2-acetylaminofluorene (AAIF)¹¹. A native DNA sample modified by the fluoro-derivative (DNA-AAAF) showed almost the same behaviour towards S₁ as DNA-AAF (Fig. 1a). On the other hand, hydrolytic attack on a DNA sample modified to the same extent with the iodo-derivative (DNA-AAIF) was much slower (Fig. 1). These results fit very well with those obtained previously using purely physicochemical experiments^{5–8}.

How the covalently linked fluorene residues induce local opening of the double helix may be viewed schematically as

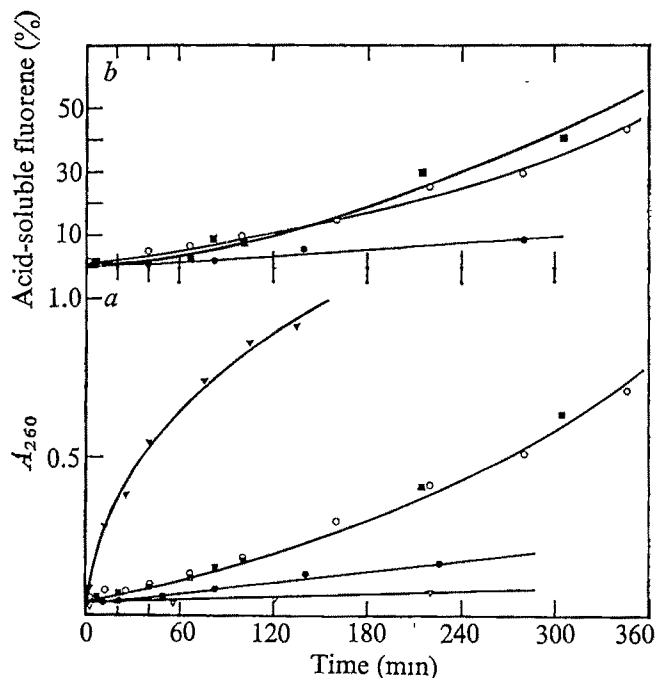


Fig. 1 Kinetics of hydrolysis of different calf thymus DNA samples by S_1 endonuclease from *Aspergillus oryzae*. ∇ , Native DNA; \blacktriangledown , heat-denatured DNA; \circ , DNA-AAFF; \blacksquare , DNA-AAFF; \bullet , DNA-AAIF. DNA was prepared from calf thymus²³ and had the following characteristics: hypochromicity at 260 nm: 40%, $S_{20,w}$: 21S; ϵ_{260} : 6,420; protein content $\leq 0.8\%$ by weight. Denatured DNA was obtained by heating a DNA sample in a sealed vial to 100 °C for 10 min. To avoid DNA renaturation the sample was rapidly chilled in ice. Reaction of native DNA samples with the fluorene derivatives was carried out as described previously⁵. All fluorene-reacted DNA samples have approximately 5% of their bases modified. Hydrolysis experiments were carried out as described by Vogt in the following buffer: 0.03 M sodium acetate, pH 4.6, 0.05 M NaCl 1 mM $ZnSO_4$, 5% glycerol. DNA concentration was about 180 $\mu g\ ml^{-1}$. Enzyme was prepared according to Vogt²². According to the definition of one unit of nuclease activity²² our enzyme solution titrates at 18 U/10 μl . *a*, Concentrations of acid-soluble material were measured as follows: incubations, carried out at 45.0 ± 0.1 °C, were terminated by chilling and addition of 0.6 ml ice-cold 10% perchloric acid to 0.3 ml aliquots. Samples were centrifuged for 15 min at 5,000 r.p.m. and A_{260} determined. *b*, Percentage of acid-soluble fluorene was determined by recording A_{305} .

follows. The ultimate carcinogen (AAAF) reacts mainly at position C-8 on guanine residues^{12,13}. If polynucleotides are strictly held in a static double-helical structure, there is no possibility of chemical reaction at position C-8 of purines (and C-6 of pyrimidines). Thus, to enable a successful carcinogen attack, we must presume a dynamic state for native DNA¹⁴, with transiently open base pairs ('breathing units'), as proposed by von Hippel *et al.*¹⁵. A consequent conformational change brings the modified base from its *anti* conformation to a *syn* conformation by rotation around the glycosyl linkage¹⁶. The three-dimensional conformation, however, is not yet clear.

To explain the local denaturation sites induced by the fluorene ring indicated by the decrease in melting temperature^{5,14,17} and by the kinetics of formaldehyde unwinding^{6,7}, we have proposed an 'insertion-denaturation model' in which the fluorene ring is accommodated between the two nearest base plates⁵⁻⁸. This model has been confirmed by Weinstein *et al.*^{9,18}. Chan *et al.*¹⁹ measured the orientation of the fluorene in renatured DNA of T4 phage and found a value consistent with the AAF residue being constrained to lie along the helix-winding angle. I have, however, obtained measurements of electric dichroism showing the fluorene ring perpendicular to the helix axis in the case of DNA-AAFF and DNA-AAFF (R.P.P.F, unpublished). On the other hand, for the iodo-derivative we found the angle

between the transition moment of fluorene at 305 nm and the helix axis to be 60°.

We may distinguish therefore two different addition products: denaturing addition products (DNA-AAF and DNA-AAFF), in which the fluorene ring gives rise to single-stranded-like regions; these regions are S_1 -sensitive; non-denaturing addition products (DNA-AAIF), in which the insertion of the fluorene ring is probably hindered (in this case by the bulky iodine atom). As local helix opening does not occur, little or no hydrolysis by the S_1 nuclease is detected. Work on the chemical nature of the addition products in DNA-AAFF and DNA-AAIF is in progress.

I stress the increased importance in chemical carcinogenesis of the reaction of cells to limit damage to DNA. Cellular repair mechanisms are a consequence of the local distortion or breakage of DNA strands²¹. Enzymes able to recognise and to excise distorted regions of DNA are probably involved in the earliest step of cellular repair.

At present there is no evidence for a correlation between the presence of locally disorganised groups of nucleotides in native DNA and the carcinogenic process. There is, however, a striking correlation between carcinogenic power of aromatic amides and the 'molecular thickness' of their aromatic ring²⁰. In most cases, if the aromatic ring is either non-planar or substituted by a bulky atom or group, the corresponding amide shows little or no carcinogenic activity.

Although AAF was essentially inactive as a carcinogen, it does not necessarily follow that AAAIF is also non-carcinogenic. The parent compound may not be 'activated' (that is, N-hydroxylated and esterified) *in vivo*. Direct tests on the carcinogenicity of AAAIF are now in progress.

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Localisation of satellite DNA sequences in nuclei and chromosomes of two plants

MANY eukaryotic DNAs can be resolved into two or more components by centrifugation to equilibrium in neutral $CsCl^{1,2}$, the minor components being called satellite DNAs. In addition to $CsCl$ satellites, minor portions of the genome have been

resolved from the main DNA band by differential binding with Ag^+ or Hg^{2+} ions, followed by centrifugation in Cs_2SO_4 .

The satellite DNA in mouse, isolated as either a CsCl satellite or as a Ag^+ complex, has been localised, by *in situ* hybridisation, over the chromocentres in interphase nuclei, and at the centromeres of all the metaphase chromosomes^{3,4}. Further satellites have been investigated in various animal species including *Drosophila*^{5,6}, *Rhynchosciara*⁷, the higher apes and man⁸. The small size of the chromosomes in plants containing CsCl satellite DNA⁹ has frustrated attempts to localise these sequences cytologically. We describe here the properties of a $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ satellite DNA from *Scilla sibirica*, a plant with large, morphologically distinguishable chromosomes containing heterochromatin¹⁰, and demonstrate by *in situ* hybridisation the nuclear and chromosomal location of these sequences. Some preliminary observations are presented for an $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ satellite DNA isolated from *Vicia faba*.

The nuclear DNA of *S. sibirica* forms a slightly asymmetrical band with a buoyant density of 1.701 g cm^{-3} on centrifugation in CsCl (Fig. 1a). When the DNA is complexed with Ag^+ ions a light satellite, comprising 5–10% of the total DNA is completely resolved from the bulk of the DNA on centrifugation in Cs_2SO_4 (Fig. 1b). After purification of this band on preparative $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ gradients the satellite DNA forms a single homogeneous band at 1.705 g cm^{-3} in CsCl (Fig. 1c). This $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ satellite DNA probably accounts for the asymmetry of the total DNA band in Fig. 1a.

Figure 2a shows the thermal denaturation curve of *S. sibirica* satellite DNA in $0.25 \times \text{SSC}$ (0.04 M NaCl and $0.004 \text{ M Na-citrate}$, $\text{pH } 7.2$). The profile reveals at least three components of very different C+G composition, separable by thermal denaturation but apparently similar in buoyant density in CsCl . The major melting component (60%), with a T_m of 91.5°C in $0.25 \times \text{SSC}$, corresponds to a high C+G composition of about 75%. Figure 2b shows that the renaturation kinetics of the satellite in $0.25 \times \text{SSC}$ are at least biphasic. Estimation of the second order rate constant for the fast component, assuming that this contributes 60% of the total satellite DNA, gives a value of $780 \text{ mol}^{-1} \text{ s}^{-1}$ in $0.5 \times \text{SSC}$. This is very similar in

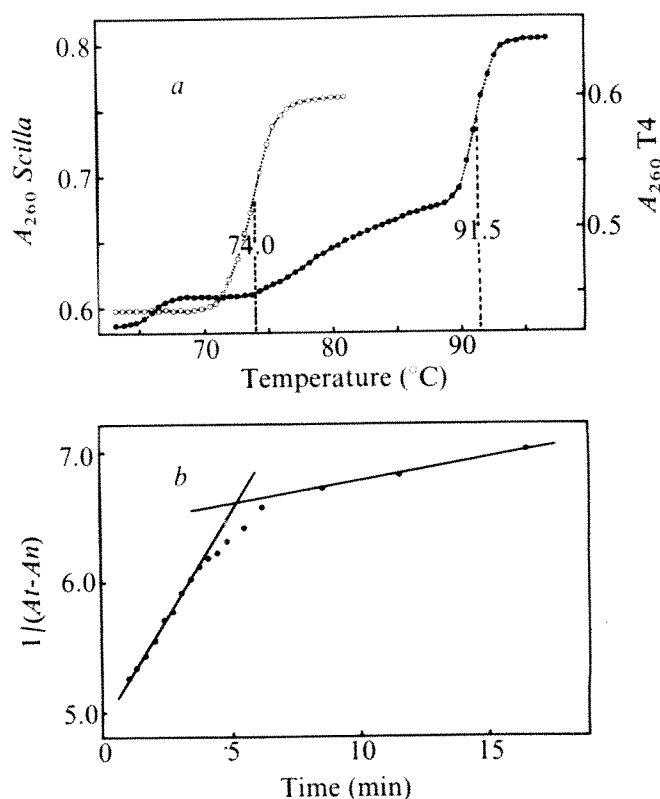
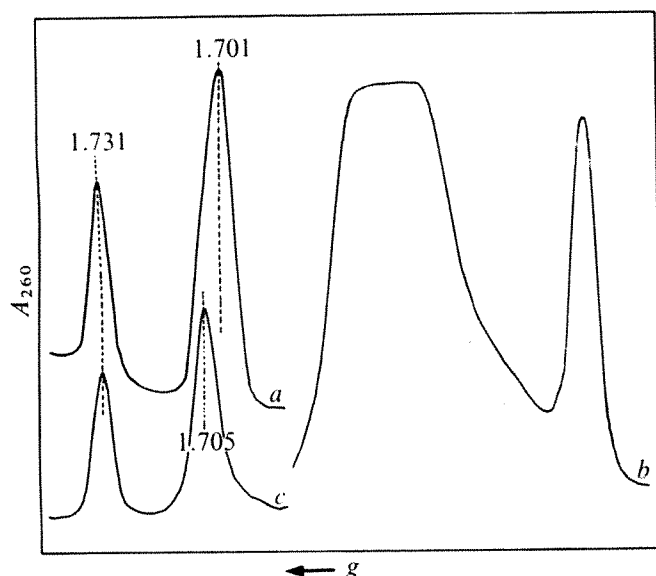


Fig. 2 a, Thermal denaturation of *S. sibirica* satellite DNA. Absorbance readings of T4 (○) and purified *S. sibirica* satellite DNA (●) in $0.25 \times \text{SSC}$ were taken at intervals in a Unicam SP 8000 spectrophotometer as the temperature control block was raised 1°C per 4 min. b, Renaturation of *S. sibirica* satellite DNA. Purified satellite DNA in $0.25 \times \text{SSC}$ was denatured at 100°C for 5 min, and then renatured at 70°C . The reciprocal of the remaining hyperchromicity ($1/(A_t - A_n)$, where $A_n = A_{260}$ of renatured DNA at time t) was plotted against time.

Fig. 1 Microdensitometer scans of ultraviolet photographs of total DNA from *Scilla sibirica*. DNA was prepared from young leaf tissue and analysed in a Model E analytical ultracentrifuge at 44,000 r.p.m. at 25°C for 20 h (ref. 19) a, $3 \mu\text{g}$ total DNA in $\text{CsCl} + 1 \mu\text{g}$ *Micrococcus lysodeikticus* marker DNA (1.731 g cm^{-3}); b, $20 \mu\text{g}$ of total DNA in Cs_2SO_4 with 0.3 M ratio of Ag^+ to DNA phosphate, $\text{pH } 8.0$; c, $3 \mu\text{g}$ of pure satellite DNA from the $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ gradient $+ 1 \mu\text{g}$ of *M. lysodeikticus* DNA in CsCl .



complexity to mouse satellite DNA and the fast component of melon satellite DNA¹¹.

Figure 3a–e shows photomicrographs of autoradiographs of cytological preparations made after hybridisation with complementary RNA (cRNA) transcribed *in vitro* from pure satellite DNA of *S. sibirica*. The grains are clearly localised at interphase (Fig. 3a) over the conspicuous chromocentres characteristic of the species^{10,12}. The label is 24 times more concentrated over the heterochromatin than the euchromatin, a factor which is not accounted for by the different density of DNA in heterochromatin, which is only 2.5–3 times^{13,14} more concentrated than the euchromatin. At metaphase (Fig. 3b–d) clusters of grains appear over restricted regions of the chromosomes. The longest homologous pair (1, Fig. 4b) and one of the shorter pairs (5) have grains restricted to the end of one arm. Another pair (2) is similar but in four of 18 such chromosomes examined grains were localised at both ends. The third pair contained relatively few grains, with localisation occurring in only four of 18 chromosomes examined. The fourth pair showed clear distal and interstitial grain clusters and the sixth showed localisation either at one end of the short arm or interstitially on the long arm, with about equal incidence. This may be explained by heterochromatic (H) segment heterozygosity¹⁵ or chromosome inversion. This distribution of satellite DNA corresponds closely with the H segments as revealed by cold treatment¹² (Fig. 4a). These H segments are stained by Geimsa¹⁶, and appear as bands of low fluorescence after treatment with quinacrine mustard or quinacrine¹⁷.

There are usually about twice as many grain clusters and H segments in the metaphase chromosomes as in the interphase

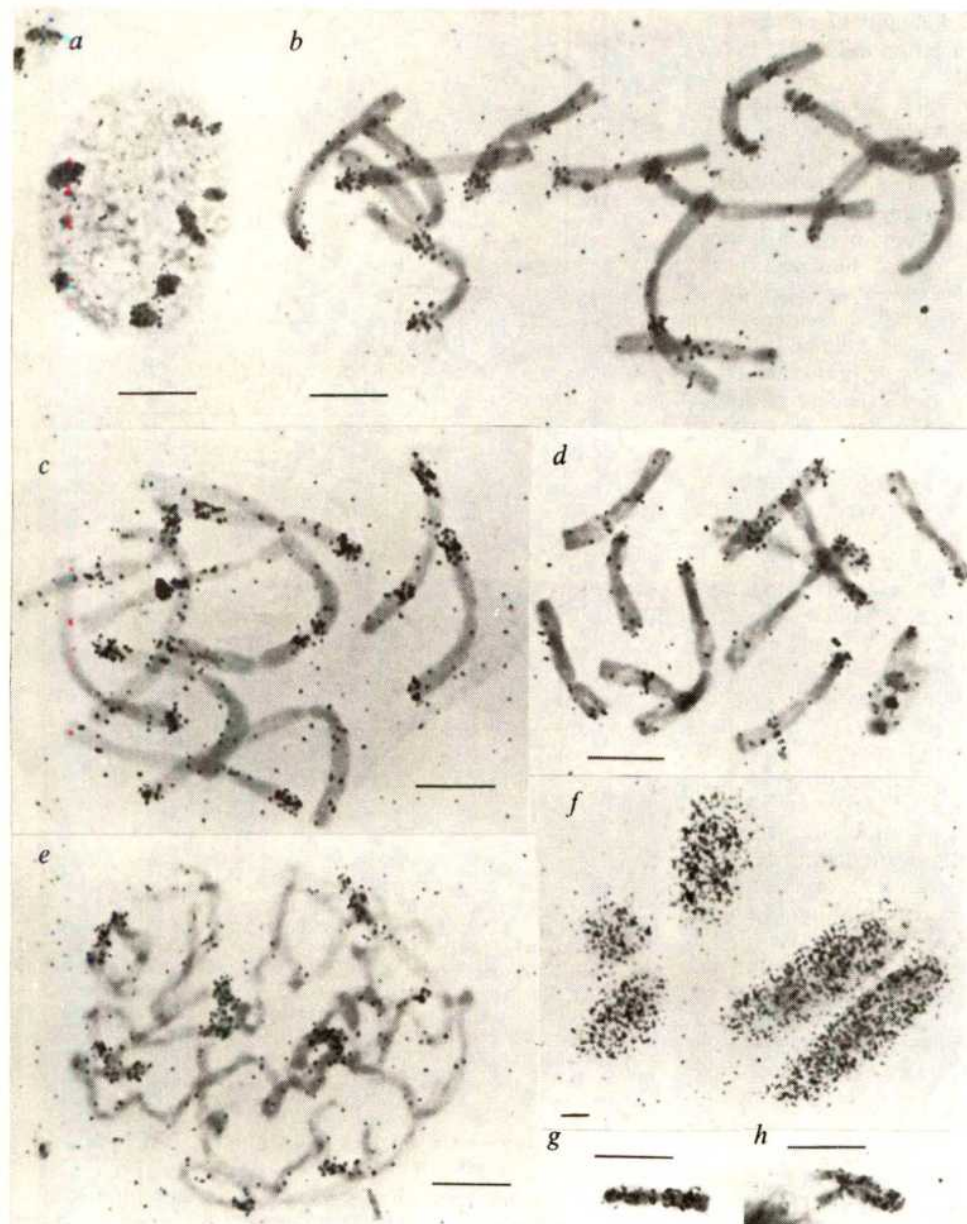
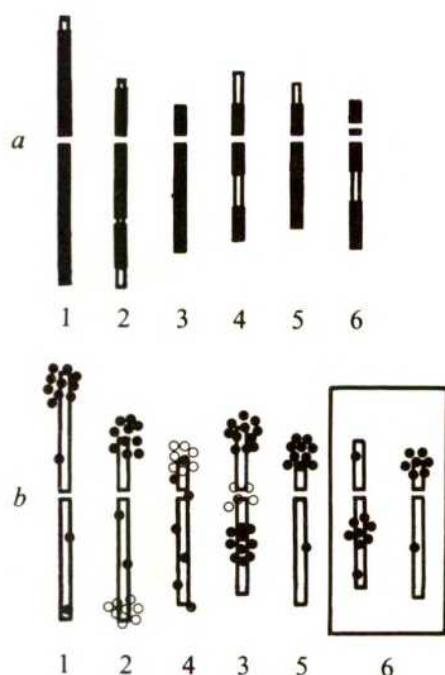


Fig. 3 *In situ* hybridisation of *S. sibirica* and *V. faba* satellite DNAs. RNA complementary to pure satellite DNA was transcribed *in vitro* using all four ^3H -nucleoside triphosphates²⁰. Colchicine-treated roots were fixed in 3:1 ethanol-acetic acid for 1 h at room temperature followed by further treatment with 45% acetic acid for 24 h. The root tips were squashed on to slides, the coverslips removed, and the preparations denatured in $0.1 \times \text{SSC}$ at 100°C for 0.5 min. After dehydration and air drying, the slides were hybridised with cRNA (specific activity 10^8 d.p.m. μg^{-1} , $2 \mu\text{g cm}^{-2}$) for 5.5 h at 75°C in $6 \times \text{SSC}$. The slides were washed in $2 \times \text{SSC}$, treated with RNase and extensively washed in $2 \times \text{SSC}$. After dehydration and drying the slides were coated with Ilford K2 photographic emulsion and exposed in a light-proof, desiccated box at 4°C for 5 weeks. The preparations were developed in Kodak D19b developer, fixed and washed before staining with Geimsa (pH 6.9) and mounting in Euparal. *a*, Interphase nucleus of *S. sibirica* showing localised labelling of the chromocentres. *b, c, d*, Metaphase cells of *S. sibirica* ($2n = 12$) showing mainly distal localisation of the hybridised cRNA. *e*, Prophase of *S. sibirica*. *f*, Interphase nuclei of *V. faba*. *g, h*, Individual chromosomes of *V. faba*. The bars represent $10 \mu\text{m}$.



or prophase nuclei, (Fig. 3e), suggesting a link between repeated satellite sequences and somatic chromosome association and mechanics¹⁸.

In situ hybridisation of an $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ satellite DNA from *Vicia faba* is also shown in Fig. 3f-h. In this instance the satellite sequences appear to be more generally spread over the nuclei (Fig. 3f), and over the chromosomes (Fig. 3g,h). There is a suggestion that the ends of the chromosome arms are unlabelled and, in addition, certain proximal regions. It is interesting that, in contrast to *S. sibirica*, most of the *V. faba* chromosome shows low fluorescence after quinacrine treatment¹⁷, suggesting a possible correlation with the satellite DNA sequences.

We conclude that the satellite DNA of *S. sibirica* is highly localised at positions which closely correspond to those of the cold sensitive heterochromatin. Although this heterochromatin

Fig. 4 Idiograms of *S. sibirica* ($2n = 12$). *a*, The position of the heterochromatic (H) segments revealed by cold treatment at -12°C is indicated by open segments (after Baumann¹²). *b*, Summary of nine analyses of complete metaphase cells of *S. sibirica*, after *in situ* hybridisation, showing the positions of the grain clusters. Grain clusters always present (●), sometimes present (○). Chromosome 6 was present in our material in either of the alternative forms shown.

is in some ways different from the centromeric form observed in other species such as mouse, it also contains highly repeated satellite DNA sequences.

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Generation of discrete yeast DNA fragments by endonuclease RI

FINE structure genetic analysis has shown that the *ilv1* gene of yeast, *Saccharomyces cerevisiae*, is multifunctional¹⁻³. The *ilv1* gene product, threonine deaminase, shows catalytic activity, as well as participating in multivalent repression of other enzymes involved in isoleucine-valine biosynthetic pathways¹⁻³. A better understanding of the regulatory role of the *ilv1* gene product and other proteins, such as isoleucyl-tRNA synthetase, which in addition to the *ilv1* gene product seems to regulate *ilv2* and *ilv3* gene expression, could be obtained if a pure preparation of the various *ilv* genes were isolated in large quantities. One way of isolating the genes would be to use restriction enzymes to cleave yeast DNA which contains normal *ilv* genes and join the fragments to a bacterial plasmid. Because of the similarity of the isoleucine-valine pathways in yeast and *Escherichia coli*, the fused DNA could then be transformed into a strain of *E. coli* with a deletion in the particular *ilv* gene. Restriction enzyme-cleaved DNA from various sources has been amplified by growth in *E. coli*⁴⁻⁷. We have found that restriction enzyme *EcoRI* treatment of yeast DNA results in not only a reduction in the size of total DNA but also the generation of several distinct species of homogeneous size DNAs some of which are derived from ribosomal genes and others from mitochondrial genes.

DNA isolated from *S. cerevisiae* MAR-33 (ref. 8), although heterogeneous, was as large as 20×10^6 – 30×10^6 daltons. It migrates parallel to DNA of phage T3 and λ (molecular weight 23×10^6 and 31×10^6 , respectively) in 1% agarose. After treatment with *EcoRI* the yeast DNA profile was reduced in size but showed discrete bands. As Fig. 1 shows, at least ten distinct bands could be identified in a complete *EcoRI* digest of yeast DNA. Of these, the first and the last band was also present in untreated DNA. Thus, *EcoRI* treatment generated at least eight groups of homogeneously sized DNA fragments. More bands appeared in conditions of limited *EcoRI* digestion (Fig. 2). These seemed to be precursor, heavier DNA fragments that are found when *EcoRI* concentration is limiting (Fig. 2a) or the

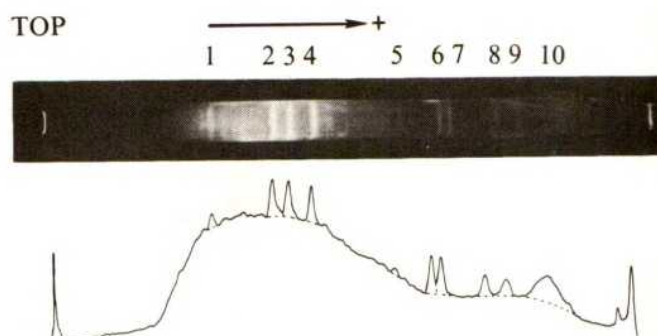


Fig. 1 *EcoRI*-yeast DNA fragments appearing as discrete bands. 100 μ g of yeast DNA was treated with 5 μ l of the restriction enzyme *EcoRI* for various intervals at 37 °C. Above is an electrophoresis pattern of 10 μ g of *EcoRI* DNA (3 h incubation with *EcoRI*) in 1% agarose gel containing 0.5 μ g ml⁻¹ of ethidium bromide and run at 4 mA per gel for 2.5 h at 24 °C. The negative photograph of the gel was run through a scanner and is presented below the picture of the gel.

digestion is carried out for a short interval, such as 2 min (Fig. 2b). These intermediates disappeared in about 6 min at 37 °C when *EcoRI* was not limiting.

The ten major bands were next compared with the sizes of *EcoRI*-cleaved fragments of phage λ DNA (Fig. 3). The designated molecular weights of these fragments—13.7, 4.7, 3.7, 3.5, 3.0 and 2.1×10^6 —show that all eight *EcoRI*-cleaved yeast DNA fragments are below 2×10^6 (Table 1). When the proportion of these DNA fragments was calculated from the areas under the peaks of the gel scan in Fig. 1 the minimum number of DNA copies in each band could be estimated (Table 1). The two DNA bands present in the untreated yeast DNA gave the two extreme values. While the heaviest band, 4.1×10^6 daltons, was present in at least 10 copies, the smallest band, 1.6×10^6 daltons, was present in at least 1,900 copies. Three *EcoRI*-cleaved yeast DNA bands were present in at least 100 copies (1.0×10^6 – 1.6×10^6

Fig. 2 Heavier intermediate bands generated by *EcoRI* treatment on yeast DNA. *a*, The experiments were performed as reported in the legend to Fig. 1 with 15 μ g of yeast DNA and various concentrations of *EcoRI*. Nearly 8 μ g of this DNA was run on a 0.6% agarose gel at 4 mA per gel for 2.5 h. *b*, The experimental condition is that described in legend to Fig. 1.

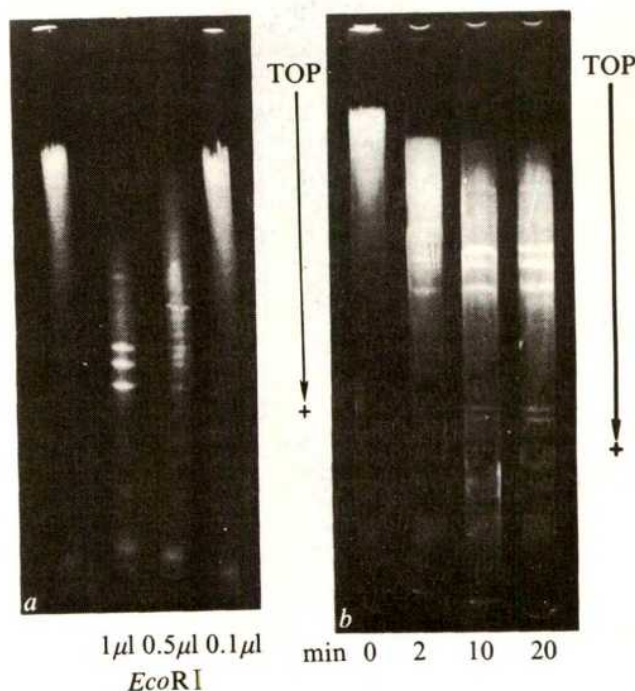


Table 1 Estimates of number of yeast DNA copies in bands appearing before and after *EcoRI* treatment

Band no.*	Molecular weight† × 10 ⁶ daltons	Proportion of total DNA‡ (%)	No. of DNA copies¶
† 1	4.10	0.34	10
2	1.60	1.18	92
3	1.40	1.20	107
4	1.05	0.98	117
5	0.58	0.19	41
6	0.42	0.94	280
7	0.38	1.04	342
8	0.26	0.67	322
9	0.23	0.63	343
†10	0.16	2.33	1,880

* Appearance of bands in order of increasing mobility as shown in Fig. 1.

† These two bands are present before *EcoRI* treatment.

‡ Estimated from known molecular weights of λ DNA fragments in Fig. 3.

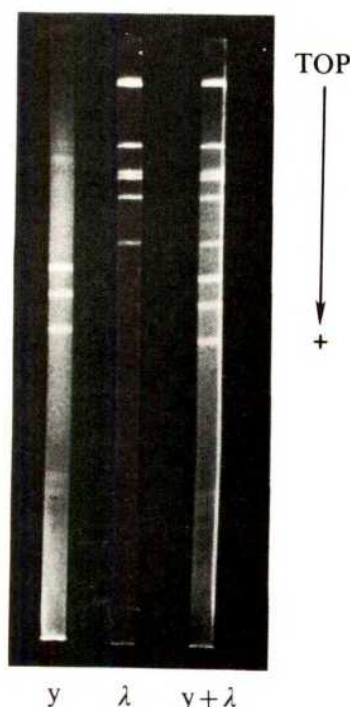
§ Obtained by measuring areas under peaks of the gel seen in Fig. 1.

¶ Calculated from a value of 1.25×10^{10} daltons for haploid yeast DNA.

daltons) and four in at least 325 copies (2×10^5 – 4×10^5 daltons). All ten bands combined constituted at least 10% of the total yeast DNA. Thus, the bulk of the *EcoRI*-cleaved yeast DNA was non-repetitive and heterogeneous in size.

Although the discrete bands of *EcoRI*-cleaved yeast DNA indicate that fragments of specific size were generated, they do not indicate whether they were of homologous sequence. There are nearly 140 copies of DNA for ribosomal RNA and 320–400 copies of DNA for transfer RNA in a haploid yeast¹⁰. Both 18S and 26S ribosomal RNA arise from a common precursor RNA molecule of 2.5×10^6 daltons (refs 11 and 12) which constitutes

Fig. 3 Size relation of *EcoRI*-yeast DNA to *EcoRI*-phage λ DNA. 10 μ g of bacteriophage λ DNA and 20 μ g of yeast DNA were digested repeatedly with *EcoRI*. 2 to 4 μ g of these *EcoRI* treated DNA individually and combined were run in a 1% agarose gel at 0.6 mA per gel for 17 h in the absence of ethidium bromide. The gels were soaked for 30 min with 5 μ g ml⁻¹ of ethidium bromide before photography.



a total ribosomal DNA of 400×10^6 – 700×10^6 daltons (ref. 13). Most of the 400×10^6 – 450×10^6 daltons of chromosome I codes for nearly 70% of the total ribosomal RNA^{14–16}. Bands 2, 3 and 4 of *EcoRI*-cleaved yeast DNA also constitute a total molecular weight of 420×10^6 daltons (Table 1). But then each of the 17–20 yeast chromosomal DNAs is of this molecular size—between 400×10^6 and 600×10^6 daltons (refs 13 and 17).

To determine which of the ten bands contained ribosomal DNA, 18S and 26S ribosomal RNA were purified from isolated ribosomal subunits and hybridised¹⁵ with DNA extracted from four segments of the complete gel and then from each of the *EcoRI*-generated bands. As Table 2a shows, the bulk of the hybridisation occurred in that segment of the gel which consisted of bands 2, 3 and 4 (Fig. 1). DNA isolated from bands 2 and 4 predominantly hybridised with 18S rRNA whereas the hybridisation with 26S rRNA was restricted to band 2 (Table 2b). Thus band 4 contains 18S rDNA. The hybridisation with band 2 indicates that this band either contains both 18S and 26S rDNAs or that the 18S RNA preparation contains some 26S RNA. In any event, a minimal assignment of 26S rDNA to DNA fragments in band number 2 and of 18S rDNA to band number 4 is possible.

Table 2 Hybridisation of yeast ribosomal RNA with *EcoRI*-generated yeast DNA fragments

<i>Eco</i> RI-generated yeast DNA isolated from gel of Fig. 1	Hybridised with radioactive	
	18S (c.p.m.)	26S
(a) Gel sections		
(i) From top to before band 2	211	135
(ii) Band 2 to 4	1,053	903
(iii) Between band 4 and 6	294	126
(iv) From band 6 to 9	328	212
(b) Band no.		
2	748	1,058
3	153	127
4	562	110
6 & 7	204	87
8 & 9	197	130

³H-ribosomal RNA (specific activity 0.3×10^6 c.p.m. μ g⁻¹) was isolated from yeast cells grown in the presence of ³H-adenine. The 60S and 40S ribosomal subunits were separated and the two RNA species were prepared by sedimentation through SDS-sucrose gradients. DNA fragments were eluted from various portions of the gel shown in Fig. 1 and immobilised on nitrocellulose filters. Low temperature hybridisation in the presence of formamide was carried out on these DNA-loaded filters with either 18S (4 μ g ml⁻¹) or 26S (4.5 μ g ml⁻¹) rRNA by a method very similar to that of Finkelstein *et al.*¹⁵. Nonspecific binding of ³H-rRNA to blank filters gave a value of 15 c.p.m. above background and has been subtracted from the above values. The values reported are the averages of duplicates.

The contribution of mitochondrial DNA in generating these fragments could be quite significant. Analytical ultracentrifugation has shown that the yeast DNA preparation contains between 5 and 6% of mitochondrial DNA. This alone would not account for all the yeast DNA bands. Furthermore, yeast DNA preparations isolated after isopycnic centrifugation in CsCl showed similar bands after treatment with *EcoRI* all throughout the gradient profile. This suggests that the bands are not exclusively of mitochondrial DNA (light density)¹⁸ or ribosomal DNA (heavy density)^{18–20} in origin. Bands 6 to 9 could be mitochondrial, however, since they are absent when yeast nuclear DNA separated from mitochondrial DNA by CsCl equilibrium centrifugation is subjected to *EcoRI* digestion and applied to the 1% agarose gel.

In conclusion, most yeast DNA fragments generated by *EcoRI* treatment are not highly repetitive, although several classes of homogeneously sized DNA species are found. Some of the

enriched DNA species seem to be mitochondrial while two size classes contain ribosomal DNA sequences. The easy availability of DNA sequences such as those for the ribosomal genes should enhance the development of the methodology for the isolation of the *ilv* structural genes.

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Determination of 5'-triphosphate terminus of 16S ribosomal RNA precursor

THE genes for the 23S and 16S rRNAs are physically linked¹. Using the drugs rifampicin and actinomycin D, it has been shown that the 23S and 16S rRNA cistrons are co-transcribed and the promoter is proximal to 16S rRNA cistron^{2,3}. Recent work with an *Escherichia coli* strain deficient in RNase III has provided further evidence for co-transcription, showing the presence of a long polycistronic RNA which could be cleaved to the immediate precursors of 23S and 16S rRNAs when treated with RNase III *in vitro*⁴. We have now studied the transcription of rRNA in *E. coli* using γ -³²P-labelled triphosphates and have shown that the 16S rRNA precursor in our preparation has a 5'-pppA end and that in our system it is neither cleaved nor replaced by 5'-pA as observed by Takanami⁵. We have not found any detectable 5'- γ -³²P-triphosphate end in the 23S rRNA species. This observation further verifies directly that both the 16S and 23S rRNAs belong to one transcriptional unit in the following order: promoter–16S–23S.

RNA transcription proceeds in the 5'→3' direction and the first nucleotide at the 5' end has so far been found to be a purine⁶, either ATP or GTP. We used γ -³²P-ATP and γ -³²P-GTP to study the initiation of rRNA transcription in *E. coli* spheroplasts. The presence of radioactivity in the nucleotide triphosphates of the RNA hydrolysate would represent incorporation of the label at the 5' end of the RNA. The advantage of using *E. coli* spheroplasts is that there is insignificant

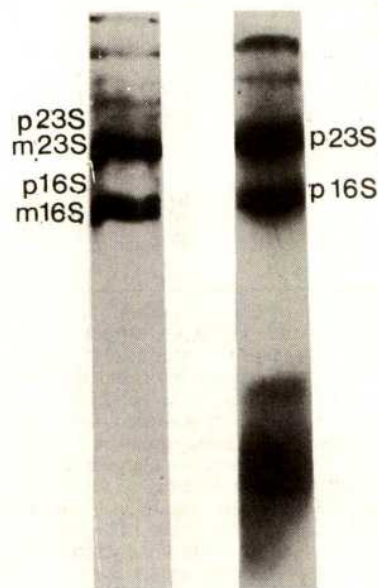


Fig. 1 *E. coli* CP78 (*his*⁻, *leu*⁻, *thr*⁻, *arg*⁻, *thi*⁻, *rel*⁺) was grown in 20 ml minimal M9 medium until absorbance reached 0.1 (2×10^8 cells ml⁻¹). The culture was then spun down at 6,000 r.p.m. for 10 min at room temperature. The pellet was resuspended and shaken in 20 ml of a plasmolysing buffer containing 10⁻³ M EDTA, 0.12 M Tris, pH 7.8 for 3 min. This rendered the cells permeable to nucleotide triphosphates for no less than 15 min. The cells were then poured into 20 ml pre-warmed M9 minimal medium containing 0.5 mCi γ -³²P-ATP or 0.5 mCi γ -³²P-GTP (New England Nuclear, specific activity containing less than 0.1% of α -³²P-ATP and α -³²P-GTP as well as orthophosphate. γ -³²P-GTP has a specific activity of 0.0552 mCi mol⁻¹ and γ -³²P-ATP of 0.0316 mCi mol⁻¹). This labelling procedure was terminated after 15 min by pouring the culture into 5 ml frozen M9 buffer, and immediately spun down at 4 °C. RNA extraction was then carried out by resuspending the pellet in 25% sucrose, 0.01 M Tris, pH 7.2, and then following the hot phenol technique described in ref. 8. The RNA extract was precipitated by adding 0.1 ml ml⁻¹ of 3 M sodium acetate, and 2 volumes of cold ethanol, and left overnight at -20 °C. The precipitate was spun at 10,000 r.p.m. for 20 min, and resuspended in 1 ml of 0.01 M sodium acetate buffer, pH 5.1. The RNA (total) was analysed through a 5 cm, 1.2% polyacrylamide-agarose gel, and electrophoresed at 5 mA and 50 V for 1.75 h. The gels were sliced and exposed to X-ray sensitive films and developed.

background due to pyrophosphate exchange and little breakdown of triphosphates to monophosphates. Thus incorporation of nucleotides with phosphate labelled at the α -position is negligible.

In this system, the transcription is qualitatively normal, as evidenced by our separate studies on ³H-UTP uptake and titrations of *lac* or *gal* mRNA. The rate of RNA synthesis is, however, reduced to 30%. This was also observed by others⁷. The nucleases responsible for processing the 16S and 23S precursors in this system become very inefficient, as shown in autoradiograms of RNA isolated from spheroplasts and subjected to polyacrylamide gel electrophoresis (Fig. 1). As Fig. 1 shows, the autoradiogram of the normal rRNAs in *E. coli* has four bands. From top to bottom of the gel they are the precursor 23S (p23S), matured 23S m23S, precursor 16S (p16S) and mature 16S (m16S) RNA. As for the rRNAs from the *E. coli* spheroplasts only the 23S and 16S precursors are seen.

RNAs labelled with γ -³²P-nucleotide triphosphate were isolated from *E. coli* spheroplasts. They were fractionated into p23S and p16S by sucrose gradient and digested by alkali treatment (see footnote of Table 1). The hydrolysates from p23 and p16 were then spotted on thin-layer chromatographic sheets

Table 1 Radioactivity from the alkaline hydrolysates of rRNAs isolated from *E. coli* CP78 spheroplast

³² P-labelled purine nucleotides	Radioactivity (c.p.m.) obtained from TLC spots with rRNA samples*	
	p16S†	p23S†
³² pppAp	367	0
³² pppGp	0	0
AMP ³²	187	386
GMP ³²	146	337

* γ -³²P-labelled ATP or GTP was used to label RNA isolated from *E. coli* CP78 spheroplasts. The RNA extract (for method see legend under Fig. 1) was subjected to a linear 5–20% sucrose gradient centrifugation at pH 5.1, using SW 40 rotor and Beckman ultracentrifuge L5-40 at 4 °C and centrifuging for 12 h at 25,000 r.p.m. The fractions containing p23S and p16S RNAs were collected and their specific activities determined by A_{260} (410 and 487 c.p.m. μg^{-1} , respectively). These two species of rRNA were first dialysed in distilled water for 6 h to eliminate sucrose, and then hydrolysed separately by treating with 0.3 M KOH at 37 °C for 16 h. The hydrolysate contains nucleotide tetraphosphates and monophosphate⁶. The hydrolysates were then neutralised by adding appropriate volumes of 1 M perchloric acid. The precipitate, potassium perchlorate, was pelleted by centrifugation at 12,000 r.p.m. for 20 min in a Sorvall RC2B centrifuge at 4 °C. Fractions of the supernatant (600 c.p.m. each or approximately 1.5 μg) were spotted on and analysed by thin-layer chromatography using plastic backed sheet obtained from Macherey-Nagel, Polygram cel 300 PEI. Non-radioactive ATP AMP, GTP and GMP were also spotted on the same sheet as reference markers. The chromatographic sheet was run in 1 M LiCl, 1 M acetic acid for 2.5 h at 4 °C. The various spots could be identified by exposing the sheet under a mineral ultraviolet lamp. The tetraphosphates and monophosphates spots were cut out carefully, dried at room temperature for 2 h and then counted on a Packard scintillation counter (Model 3375, Tri-Carb) in toluene-based Omnifluor.

†p16S and p23S refer to precursors of 16S and 23S rRNA species.

and separated. The results are tabulated in Table 1. There is a conspicuous tetraphosphate spot for p16S RNA hydrolysate that is not detectable in the case of p23S rRNA hydrolysate. Therefore, for the p23S rRNA hydrolysate, the region corresponding to the tetraphosphate spot was located by adjusting with the 16S rRNA spot by eye estimation, and a considerable area of the TLC around the region was cut. Lines 1 and 3 of column 4 (Table 1) represent the radioactivity (c.p.m.) of the tetraphosphate spots for p16S (precursor of 16S rRNA) and p23S (precursor of 23S rRNA) rRNA hydrolysates, which are 367 c.p.m. and 0 c.p.m. respectively. The first and third line in Table 1, column 5 are the radioactivity due to XM³²P spots of p16S and p23S hydrolysates.

Table 1 indicates that there is a breakdown of the γ -³²P-triphosphate label, but the extent is not very high. Considering the specific activity of γ -³²P-ATP (2.5 $\mu\text{Ci ml}^{-1}$) used for labelling rRNAs and the fact that only one 5'-³²P-labelled ATP would appear on the 5' end of each p16S RNA molecule the amount of ³²P-monophosphate, incorporated along the length of p16S or p23S rRNA is calculated as less than 0.0001% of input radioactivity. Incorporation of the labelled phosphorus into the entire length of the rRNA molecules is presumably the reason for the presence of approximately double the number of c.p.m. in the monophosphate spots of p23S compared with that of p16S RNA. γ -³²P-nucleotide triphosphates were incorporated into the p16S rRNA but not into the p23S rRNA. Because the first nucleotide incorporated always retains its triphosphate ends, we can minimise the possibility of pyrophosphate exchange into the triphosphate position. Therefore any radioactivity present in the tetraphosphate spots would be the γ -labelled ³²P-nucleotide triphosphates.

To verify which of the two purines (γ -³²P-ATP or γ -³²P-GTP) was incorporated as the first nucleotide, similar experimental procedures were repeated using only γ -³²P-GTP. Results are tabulated in Table 1, lines 2 and 4. Tetraphosphate spots of both p16S and p23S RNA hydrolysates contained no detectable radioactivity. This suggests that GTP was not the

initiating nucleotide triphosphate and therefore ATP must be the first nucleotide incorporated.

We therefore conclude, since the p16S rRNA hydrolysate contained ³²P-radioactivity in its tetraphosphate spot and p23S rRNA did not, that: (1) initiation of rRNA transcription can only take place in the 16S rRNA gene, (2) ATP is the initiating nucleotide, and (3) transcription initiating at 16S rRNA proceeds towards 23S rRNA without further reinitiation.

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Corrigenda

In the article "Collimation of auroral particles of time-varying acceleration" by D. S. Hall and D. A. Bryant (*Nature*, **251**, 402; 1974) there is an error in equation (10). The denominator should read $\sqrt{2(\sigma/E_0)}$ and not as printed. Also the value of A in the legend to Fig. 1 should read $9.1 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ keV}^{-1}$ and not as printed.

In the article "Oxidation of nickel by nitric oxide as a new strong oxidant" by Y. Takasu, Y. Matsuda, S.-I. Maru and N. Hayashi (*Nature*, **255**, 544; 1975) there are several errors in the figures and legends. On the ordinate of Figs 2 and 3, for K_1 read K_1' . The legends to Figs 2 and 3 should read:

Fig. 2 Relationship between K_1' ($\text{g cm}^{-2} \text{ s}^{-1}$) and temperature ($P_{\text{NO}} = 10 \text{ mmHg}$). $E = 17.8 \text{ kcalorie}$.

Fig. 3 Relationship between K_1' ($\text{g cm}^{-2} \text{ s}^{-1}$) and the pressure of nitric oxide (700 °C). $n = 0.74$.

Errata

In the article "Excision of thymine dimers from specifically incised DNA by extracts of xeroderma pigmentosum cells" by K. Cook, E. C. Friedberg and J. E. Cleaver (*Nature*, **256**, 235; 1975) the following corrections should be made. In line 3 of the legend to Table 1, for 50–100 erg mm^{-2} read 5–10 J m^{-2} . The list of authors should include as the last author H. Slor, Department of Human Genetics, University of Tel Aviv, Israel. The asterisk after J. E. Cleaver should indicate that his address is the Laboratory of Radiobiology, University of California School of Medicine, San Francisco, California.

In the article "Pathogenicity and cerato-ulmin production in *Ceratomyces ulmi*" by S. Takai (*Nature*, **252**, 124; 1974) there is an error in the second sentence of paragraph 6. For (120 mg ml^{-1}) read (120 $\mu\text{g ml}^{-1}$).

matters arising

Formaldehyde polymers in interstellar space

THE suggestion¹ that polyoxymethylene (POM) crystals exist in interstellar space must be reviewed in terms of the thermodynamics of polymer-monomer equilibria². The formaldehyde-POM equilibrium is notoriously mobile³, unless the polymer is end-capped⁴. This treatment is applied to commercial samples such as those whose infrared spectra were reported by Tadokoro *et al.*⁵, which provided data for the argument¹ for POM in space.

The partial pressure of formaldehyde in equilibrium with POM has been determined over a small temperature range⁶, and, by extrapolation, the partial pressure at 20 K (the temperature at which the polymer is supposed to form¹) is equivalent to $2 \times 10^{-14.3}$ molecules cm^{-3} . Chemical thermodynamics thus supports the hypothesis that the polymer exists in some regions of space: a mechanism for initiating the polymerisation process on the surface of mineral particles may be provided by ionising radiation, though the rate of propagation must be extremely small at such a temperature.

At 445 K, the temperature assumed for the dust in the Trapezium nebula¹, the equilibrium partial pressure of formaldehyde above POM, is more than 1 atmosphere, which is greatly in excess of any likely nebulous condition. Only if there were a means for end-capping interstellar POM would it exist at 445 K, and the survival time of such material in the ionising radiation of an H II region would be short. It is, therefore, doubtful that the infrared emission from the Trapezium nebula is from a POM source at that temperature.

It is interesting to note that the concentration of formaldehyde molecules in the Trapezium nebula seems to be greater where ionised atomic material is most dense⁷, and it is possible to postulate a role for the formaldehyde-POM equilibrium in the globular model⁸ of the core of the nebula, assuming that the temperature and formaldehyde molecule concentrations were suitable.

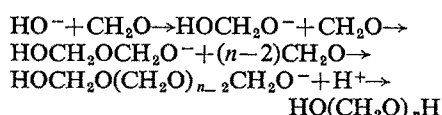
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WICKRAMASINGHE AND SANTHANAN REPLY—Preliminary remarks on the end-capping of formaldehyde polymers have already been made in the context of the formation of interstellar POM¹. The vapour pressure data for such stabilised polymers (for example, resins consisting mainly of $(\text{H}_2\text{CO})_n$) do not seem to be readily available. The extrapolations of vapour pressure^{2,3} data discussed by Fawcett⁴ relate to unstabilised POM. For a gas kinetic temperature of 100 K, as may be appropriate to an H I region, the number density of gaseous H_2CO molecules in equilibrium with unstabilised POM reaches the observed value $\sim 10^{-8} \text{ cm}^{-3}$ at a particle temperature, T , $\sim 115 \text{ K}$. As that temperature is less than the temperature of most grains, there can be no doubt about the stability of POM even if it were not suitably end-capped in normal interstellar conditions.

We consider it unlikely, however, that interstellar polyformaldehyde will not be end-capped. The formation of POM mantles or whiskers is expected to occur mainly in molecular clouds in the presence of considerable abundances of OH, H_2O and other molecules including CH_3OH . The H_2CO polymerisation could be initiated by the well established process of anionic polymerisation⁵. For example, HO^- from interstellar water molecules may have a crucial role:



End-capping which would ensure maximum stability may be achieved by a similar interaction with interstellar methanol (CH_3OH) molecules.

The polymerisation of interstellar formaldehyde could be initiated and

end-capped by this or any other process involving anionic attack. Suitably end-capped formaldehyde polymers (for example, CH_2O resins) are highly stable at room temperature. Such crystalline polymers are expected to have sufficiently low vapour pressures to ensure their stability in H II regions up to temperatures, T , of about 400–500 K, or even higher. Particles heated to temperatures T , of more than 300 K could account for the observed 8–12 band in the Trapezium nebula^{5,6}.

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Longitudinal photons in the fireball

BYRNE AND BURMAN have discussed the significance of astrophysical data for the problem of the photon mass¹. A question might arise concerning the possible presence of longitudinal photons in the microwave background were the photon mass not zero. In true thermal equilibrium, the longitudinal modes of the field would have to be populated to the same extent as the transverse modes. Bass and Schrödinger concluded some time ago that millions of years would be required for an appreciable fraction of longitudinal photons to build up in any terrestrial situation². Such time intervals, however, are available when dealing with cosmology.

The probabilities for the emission of longitudinal and transverse vector mesons with wave number k in a given process are in the ratio $m^2 c^2 / (\hbar^2 k^2 + m^2 c^2)$, where m is the meson rest mass³. When $\hbar k \gg mc$, this is approximately $(\lambda/\lambda_c)^2$, where λ is the wavelength and $\lambda_c = h/mc$. If the mean free path of the photon is L , the number of collisions per second is c/L , and the fraction of transverse photons converted

into longitudinal photons every second will be $(c/L)(\lambda/\lambda_c)^2 \equiv 1/\tau$. Also $1/L \approx n\sigma$, where n is the density of scatterers and σ the scattering cross section. We are concerned with the photons of the fireball moving through hydrogen, and can assume σ to be the cross section for Rayleigh scattering: $\sigma \approx a^2\lambda_b^4/\lambda^4$, where a is the classical electron radius and λ_b is the wavelength corresponding to the binding energy for hydrogen, about 10^{-5} cm. Both λ and n will change because of the expansion of the Universe: $\lambda = S\lambda_0$ and $n = n_0/S^3$, where λ_0 and n_0 are the quantities at epoch t_0 and S is the cosmological scale factor, chosen so that $S_0 = 1$. That gives:

$$\tau \approx S^5 \lambda_0^2 \lambda_c^2 / cn_0 a^2 \lambda_b^4$$

The factor τ depends strongly on S , which is now of the order of 1,000 if the initial epoch is that of the decoupling of the fireball radiation and matter; it is smallest when $S = 1$, $n_0 \approx 10^4$ atom cm^{-3} and $\lambda_0 \approx 10^{-4}$ cm. If $m = 10^{-52}$ g (ref. 4) then $\tau \approx 10^{44}$ yr. Thus, the fraction of longitudinal photons in the field after even many times 10^{10} yr is negligible. Even though long times are available, the free paths of photons are too long for sufficient scattering events to occur.

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¹ Byrne, J. C., and Burman, R. R., *Nature*, **253**, 27 (1975).

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Do epicentres migrate on the San Andreas Fault?

WOOD AND ALLEN¹ have suggested that seismicity on a portion of the San Andreas Fault can be used in support of a model of recurring migration of epicentres. They considered 27 earthquakes of magnitude, $M \geq 5$ within 30 km of the fault between 35.5°N and 38.5°N which occurred between 1930 and 1972. Five parallel line segments are drawn through the time-latitude plot of 27 points; the common slope of the lines is presumed to give the velocity of migration of earthquakes. Several of these events correlate with one another; any line in the neighbourhood of events that are close together in latitude and time will provide a satisfactory fit.

Obvious clustered events are the quadruplet (1,2,3,4) and the pairs (15,16; 5,6; 19,20; and 25,26) (Table 1 of Wood and Allen). Probably, other correlated events could be found were a different model used. Further, event 13 seems not to have been used in the fit.

Thus, there are 19 or fewer independent events in the data set. If a model is used in which the slopes of the line segments are fixed, then there are 19 or fewer degrees of freedom in the data, which are used to fit the intercepts of the segments.

The number of degrees of freedom in the model used by Wood and Allen may be about 15 in addition to the common slope: that is, the intercepts and end points of each of the five lines. The number of degrees of freedom in the end points is a function of the length of the segments and is difficult to evaluate. Thus, the number of degrees of freedom in the model is roughly equal to the number of degrees of freedom in the data, and we conclude that there are inadequate data to support the parametric complexity of the migration model. We believe that the apparent migration of epicentres along part of the San Andreas Fault is an artefact of Wood and Allen's model.

Wood and Allen forecast an earthquake with $M \geq 5$ in a restricted time-space interval along the San Andreas Fault. Though the complexity of the model makes it easy to give qualitative predictions, quantitative predictions in terms of probabilities remain difficult. Wood and Allen have avoided the question of estimating the risk that is: the probable number of shocks that will occur in the time-space region identified as dangerous. We can calculate the risk on the basis of a simpler model involving a considerably smaller number of degrees of freedom. Suppose that earthquakes are Poissonian between 36.75° and 37.05°N . Seven earthquakes are then found in the $25 \text{ yr} \times 33 \text{ km}$ time-space region; the area identified as dangerous by Wood and Allen is about $6 \text{ yr} \times 17 \text{ km}$. Thus, 0.86 ± 0.31 shocks can be expected in the defined time-space interval. Dropping the constraint that the earthquake catalogue has a space-time envelope, and assuming instead that shocks occurred randomly between 1930 and 1972, then 0.50 ± 0.19 shocks with magnitudes of more than five will occur in the smaller time-space interval.

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¹ Wood, M. D., and Allen, S. S., *Nature*, **244**, 213 (1973).

Competition and species abundance

HEBERT *et al.*¹ used samples of the aerial population of Macrolepidoptera, collected at light in Ontario, Canada, to advance an argument in competitive population dynamics. Although not entirely explicit, it seems to run as follows. Given the "generally conceded" initial premise that interspecific competition is greatest between closely related sympatric species, the hypothesis that species abundance is controlled by level of competition can be tested by the relationship between mean species' population density and their taxonomic distance. In other words, in a sample from a multispecies population, the mean number of individuals per species in each family (N_s) is expected to be negatively correlated with the number of species in that family (S_f); making the provisional assumption that the relationship is causal.

The general conclusion reached by Hebert *et al.*¹ from their evidence of an inverse relation between family size and the abundance of species, was as expected; "family differences . . . are due to competitive interactions." The experiment raises questions at all levels, technical, analytical, interpretative and logical and, because the issue is a central one, we will take these up in more detail elsewhere. Our present purpose is to show that results like these are not invariably obtained from samples of this kind and, therefore, the conclusion as stated is not general.

The data in Table 1 are sums of seven replicate samples, each one a single year's

Table 1 Species abundance and mean no. of individuals per species in each family at two selected sites in Britain

	Malham		Bangor	
	S_f	N_s	S_f	N_s
Noctuidae	86	69.03	79	10.00
Geometridae	75	90.51	103	27.15
Arctiidae	5	4.60	6	4.00
Notodontidae	4	9.75	2	3.50
Sphingidae	1	6.00	2	1.50
Other families*	1.6	16.63	2	2.33

* Five at Malham and six at Bangor.

accumulation of nightly subsamples of Macrolepidoptera collected at light, from Malham in northern England² and Bangor in North Wales, as part of the Rothamsted Insect Survey³ during 1966-72. These sites were selected from a much larger series because, in these particular instances, the relation between N_s and S_f is diametrically opposed to that given by Hebert *et al.*; the correlation is positive, not negative.

The use of selective samples to investi-

gate population properties of the kind involved here is not new⁴ and their validity is currently being investigated^{5,6}. So far, we can find no objection in principle to the technique, although the selection mechanism used to obtain a sample is obviously confounded with some behavioural properties, thus invalidating its use for certain purposes. In the present instance, the difference between our results and those of Hebert does not seem to be accountable to such artefacts and we do not question the validity of either set of data. Consequently, one must either reverse Hebert's "generally conceded" initial premise, or, following their logic, regard our result as establishing that abundance is positively dependent on competition in the Malham and Bangor communities.

As Hebert *et al.*¹ point out, we are not here concerned with replacement competition but with the diffuse competition⁷, resulting from common environmental interests⁴ and mortality factors, in which the theory of island biogeography⁸ has generated much interest. While we have good reason to regard neither experiment as conclusive, because both are partial, our positive result might seem to indicate the negative competition allowed for in Hutchinson's original niche concept⁹.

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HEBERT ET AL. REPLY—Since diffuse competition in Lepidoptera, as we view it, is the result of long term coevolution among lepidopterans, their food plants, predators and parasites, we would not expect these interactions to be strong in highly disturbed communities. The importance of analysing pristine communities in synecological studies of this sort has been recognised¹. The drastic alteration of the original vegetation in Great Britain probably precludes such studies. Ter-

borgh¹, for example, worked in a rain-forest wilderness area of 20,000 km², whereas the least disturbed site in the Rothamsted Insect Survey consisted of 3.2 acres of 80-yr-old regrowth². Our main study area (Perth Road, Ontario)³ was somewhere between these extremes; within a 1-km radius of the collecting site there was little intrusion of introduced plants and forest composition approached a climax condition for the area. Beyond this distance most of the landscape was similar within a radius of 10 km, although scattered farms were present. Similarly, at our Chaffey's Locks site, much of the original vegetation remains intact.

With regard to the effect of environmental disturbance on abundance patterns

Table 1 Species abundance at Glenburnie, Ontario

	No. of species	No. of individuals per species
Noctuidae	248	51.85
Geometridae	86	25.38
Notodontidae	28	7.68
Arctiidae	27	42.15
Sphingidae	17	10.65
Other families	21	30.90

Collection was at one light during the 1969-70 season and totalled 17,226 individuals.

in Lepidoptera we have data from Glenburnie, Ontario, a highly disturbed agricultural area about 20 km from the Perth Road site. These data (Table 1) resemble those of Taylor and Woiwod in so far as there is no indication of an inverse relationship between species abundance and family size such as existed at our Perth Road and Chaffey's Locks sites. In fact at Glenburnie the Noctuidae, the largest family, have the highest mean abundance, but this is largely the result of a single species represented by nearly 6,000 individuals. The apparent direct relationship between family size and species abundance found by Taylor and Woiwod may be the result of small sample size or possibly selective sample choice, for the Rothamsted Survey collections were made at 160 sites⁴, only two of which are reported here⁵. From this same standpoint we should make it clear that we have made collections at only three sites, two in the undisturbed areas described previously³ and the remaining collection at Glenburnie.

The difference between abundance patterns in disturbed and natural habitats is probably a reflection of the increased variance in patch size, characteristic of cultivated areas in which small remnants of the native vegetation persist in the midst of a floristically depauperate agroecosystem. Often the largest patches in such heterogeneous environments

represent novel vegetation resources. For example, in south-eastern Ontario the most important elements of the agricultural grassland are introduced species⁶. Assuming the ability to use such novel resources is fortuitous, then one would expect that the species using these patches would tend to belong to the larger families of the original source fauna and, other factors being equal, these species should be overwhelmingly abundant.

We feel that confirmation of the importance of diffuse competition should only be sought in the analysis of data collected in areas where human disturbance is minimal. We predict that the effects will be most pronounced in homogeneous areas of long term stability, such as tropical rainforest, where variance in patch size is low and thus where one *a priori* expects species equitability to be high.

We thank C. C. Bower for comments.

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Gravitational analogue of the magnetic force

SALISBURY AND MENZEL¹ have considered the case of a pair of charged masses separated by a distance, r , moving together at a velocity, v , in the direction perpendicular to the line joining their centres. The charges and masses were chosen so that the electrical and gravitational forces cancel one another in the moving coordinate system. Using only special relativity, Salisbury and Menzel arrived at the conclusion that the gravitational equivalent of the magnetic force is

$$\gamma^3 \beta^2 (2 - \beta^2) (G m_1 m_2 / r^2)$$

where m_1 and m_2 are the rest masses, $\beta = v/c$, $\gamma = (1 - \beta^2)^{-1/2}$, and G is the gravitational constant.

The correct special relativistic expression is arrived at as follows. The trans-

formation equations for forces are well known². The gravitational force on one of the masses, in their own reference frame, is Gm_1m_2/r^2 . In the 'laboratory' frame, with respect to which their velocity is v , the force is again along the line joining the centres of the masses and is $Gm_1m_2/(\gamma r^2)$.

An observer in the laboratory frame would find that the masses were γm_1 and γm_2 . If he calculated the gravitational force with the usual formula, he would find it to be $\gamma^2 Gm_1m_2/r^2$, which is larger than the true force by the quantity

$$X = [\gamma^2 - (1/\gamma)] Gm_1m_2/r^2$$

But X is not the gravitational equivalent of the magnetic force. If m_1 carries a charge q_1 and m_2 a charge q_2 , the magnetic force on q_2 is q_2vB_1 , where B_1 is the magnetic induction resulting from q_1 (ref. 3), or

$$\gamma\beta^2 q_1 q_2 / (4\pi\epsilon_0 r^2)$$

If q_1 and q_2 are of the same sign, the electric force on q_2 in the moving frame is repulsive, and the magnetic force in the laboratory frame is attractive. Since the gravitational force in the moving frame is attractive, the gravitational analogue of the magnetic force in the laboratory frame is repulsive. It is given by replacing $q_1 q_2 / 4\pi\epsilon_0$ by Gm_1m_2 in the expression already given, or by

$$\gamma\beta^2 Gm_1m_2/r^2 \approx \beta^2 Gm_1m_2/r^2$$

where m_1 and m_2 are again the rest masses since in the moving frame the masses are fixed with respect to one another.

The quantities γ_1 and γ_2 (see ref. 4) referred to by Salisbury and Menzel come from general relativity. They cannot be arrived at using only special relativity.

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¹ Salisbury, W., and Menzel, H., *Nature*, 252, 664 (1974).

² Lorrain, P., and Corson, D., *Electromagnetic Fields and Waves*, 225 (Freeman, San Francisco, 1970).

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MENZEL AND SALISBURY REPLY—Professor Lorrain is correct in his assertion that the gyron force between moving masses is repulsive. We show that in our paper. But, his suggestion that the transformation for magnetic forces between moving charges also applies to moving masses is incorrect. The magnetic force

between charges is proportional to the product of the equivalent currents, q_1v_1 and q_2v_2 . In our simplified presentation, v_1v_2 happens to equal v^2 . A similar identity occurs with the increase in mass caused by the kinetic energy of the motion. That increase in mass produces an extra force between the bodies. The force is proportional to the mass-current product, $m_1m_2v^2$ in our simple case, and must be distinguished from the gyron or magnetic-like repulsion.

That the magnetic force and the gyron force cannot have exactly the same coefficients should be clear from the fact that both experimentally and theoretically, electric charge is conserved under a Lorentz transformation, whereas mass increases with velocity.

The criticism that we are solving a problem in general relativity by means of special relativity is not valid. Particle accelerators demonstrate clearly that high accelerations and great mass increases occur. Thus, general relativity can be approached through special relativity; Einstein used the approach to obtain his famous law: $E = mc^2$.

General relativity theory is clearly incomplete because it predicts that mass is conserved in the same way as electric charge. The new Yilmaz theory of general relativity corrects this error and gives a conservation law of mass-energy and momentum that allows for a smooth transition between special and general relativity¹. Further, the new theory satisfies the experimental criteria.

Any form of relativity must have a firm experimental basis. The invariance of electric charge is well founded experimentally. The change of mass with velocity is known to a high degree of accuracy; it materially affects the design, operation, and observation of a wide variety of particle accelerators.

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¹ Yilmaz, H., *Nuovo Cim*, 7, 9 (1973)

Embryonic chick tibiae in steady electric fields

WATSON *et al.*¹ report that the growth rate of embryonic chick tibiae *in vitro* is enhanced by a pulsed electric field of 1,000 V cm⁻¹ but not by a static field. We suggest that there is a simple reason why no enhancement should be expected in the static case. This has to do with the ability of the cultures to sustain a field.

Any field produced within the tissue will decay exponentially with a relaxation time constant²

$$\tau = \epsilon_0 k / \sigma \\ = 8.8 \times 10^{-12} \text{ C}^2 \text{ N}^{-1} \text{ m}^{-2}$$

where σ is the conductivity of tissue, k the dielectric constant of tissue and ϵ_0 the permittivity of free space.

There seem to be no published data on the conductivities and dielectric constants of uncalcified embryonic bone, but Schwan³ has published data on the electric properties of other tissues (for example, lung, muscle and liver). Representative values at very low frequencies (1–10 Hz) are $\sigma = 10^{-3}$ mho cm⁻¹ and $k = 10^6$. It seems reasonable that values for uncalcified chick tibia should have the same orders of magnitude. Converting σ to m.k.s. we derive a relaxation time constant $\tau \approx 10^{-4}$ s. The corresponding value for a dielectric like fused quartz is in excess of 10^6 s.

This shows why enhancement of growth is not to be expected in a steady field. During the 9-d growth period *in vitro* there is no field within the bone, no charge movement within or on the bone, and so no bioelectric command signal to the bone, except at the instants when the field is switched on or off.

Schwan's values for k may perhaps be treated with caution, but assigning more typical values to the dielectric constant (say $k = 10$) gives essentially the same result—in the steady field there is no transducer mechanism available.

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reviews

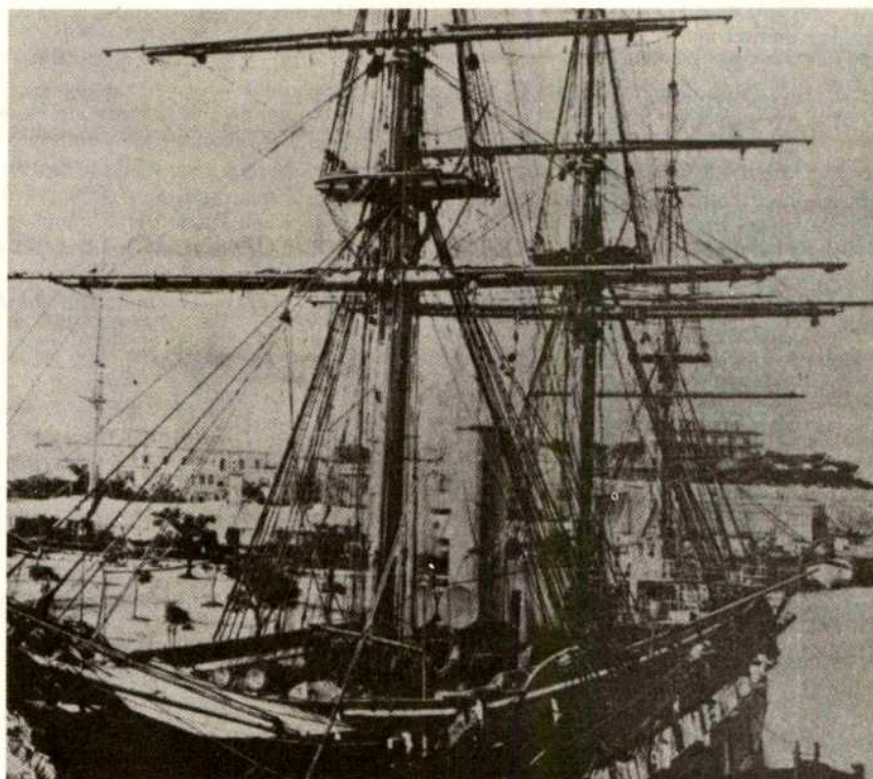
Oceanography: edge of an unfamiliar world

Courtesy Trustees of the British Museum (Natural History)

WE have become used to publishers coyly hiding the dates of publication of their books on the back of the title page; they believe, I suppose, that no one buys books when they are more than a year old. The publishers of this book* have extended the idea and concealed the fact that it is a reprint of one first published in America, by another company in 1973 (though they do say "© 1973"). This practice is dubious in relation to the purchaser; for the author, in this instance, the practice is grossly unfair. The reader looks for references to Margaret Deacon's book, which has become the standard work on the history of oceanography, and does not find them. The reason is that copies of her book only reached the US at the time that this book went to press so the author is, quite unjustly, made to seem ignorant of things one would expect to find in a book published in 1975.

The book covers the period from about 1830 to the 1970s and does not touch on the work of the 17th and 18th centuries. That is fortunate as the earlier period has been fully covered by Margaret Deacon. The opening chapters, on the early years of what eventually became the Coast and Geodetic Survey and the Hydrographic Office of the Navy, are, perhaps, the most instructive and original part of the book. There, Ms Schlee has gone back to the primary sources and one can see the back history of oddities of organisation and of disputes which lasted almost to the present time. The account of the "infamous United States Exploring Expedition" and of the shortcomings of M. F. Maury as a theorist are particularly interesting.

In the later parts of the book she demonstrates an irritating tendency to quote quite dubious and often inaccessible secondary sources when better information is easily available. For example, for an interesting quotation from



RV Challenger, one of the earliest oceanography research vessels, at Bermuda in 1873

Darwin the reader is referred to *The Darwin Reader* which contains, presumably, extracts from Darwin's works. Again, an improbable quotation from Halley is taken from *The Last Cruise of the Carnegie* where it occurs, but without any reference. Occasionally, the author is led into error by such sources: for example, Edmond Halley, with his name spelled Edmund (a form he never used in English) sails to the Antarctic in the *Paramour Pink* (a Pink is a kind of ship, not the name of a ship); both are venerable errors. The source of other errors is less clear, as when Ritter is said to have founded Scripps and to have been an "instructor in biology at the University of California at San Diego" (he was a professor at Berkeley, and UCSD was founded 60 years later by a professor from Scripps). This error is part of an eastern US and western European bias; for Ms Schlee everything west of the Ohio is Indian Country. These small errors are not of much importance but they do produce a feeling of insecurity in the reader and it is only fair to say that my doubts on several other

matters proved unfounded (for example, I doubted that St Vincent was First Lord in 1804).

The author has, perhaps, not firmly decided for whom the book is written: much of it is serious and reliable history, but in places she feels the story is getting dull and lapses into a more popular style with a wealth of irrelevant detail derived, remotely, from Hemingway and his imitators. For example: "A light snow was falling on Boston Harbour as two tugboats cast off their heavy docking lines . . ."; I am sure that she knows that it was snowing and that the lines were heavy—but that is the language of journalism rather than of history.

Perhaps I have overstressed the things that, to me, seem shortcomings; it is an interesting and readable book, contains many things that will be new to most readers and should be read by anyone with a serious interest in oceanography and the history of its development.

Some of the figures are little more than black smudges; they were only slightly better in the American edition.

E. Bullard

**A History of Oceanography: The Edge of an Unfamiliar World*. By Susan Schlee. Pp. 398. (Robert Hale: London, February 1975.) £5.

Numbers of people

Population Policy in Developed Countries. Edited by Bernard Berelson. Pp. xiii+793. (A Population Council Book.) (McGraw Hill: New York and London, 1974.) \$17.50.

THIS is a collection of essays on the present demographic situation and the policies relevant to population growth, composition and distribution in 24 'advanced' countries (including Argentina, Greece and Spain). Bernard Berelson contributes an introduction and a very sensible and comprehensive summary.

It is clear from this collection that Britain, the Netherlands and the United States are the only countries in which there has been any marked concern during the 1960s with overpopulation, and even in those countries, the concern is muted and becoming more so. In all others, the concern is more with increasing the total population or, as in the cases of Israel and South Africa, with redressing a perceived imbalance between different groups. The reasons for this are various. They include an anxiety about future manpower (even the Netherlands, although worried by high density, encourages immigration), an anxiety about political status (most evident in Argentina and Israel, but by no means absent from many other countries), and a more diffuse anxiety about the dangers of some sort of inertia from stationary or declining numbers. It is interesting that the arguments reviewed for and against rising numbers are all, without exception, inconclusive. The sanest stance is perhaps taken by the Swedes, who seem to be entirely agnostic about the reputed effects of different demographics and concern themselves instead with the maintenance and improvement of the quality of life for those who are already living.

It is also clear from these essays, however, that any country wishing to do anything about its rate of population growth is faced with considerable difficulties. Punitive policies (such as that recently introduced in Singapore) are out of the question for moral and political reasons. And less punitive policies seem to have uncertain or weak effects. There is little moral or political room for manoeuvre over mortality; providing adequate facilities for birth control is no more likely to reduce fertility than is withdrawing them likely to raise it; and although there is considerable political, if not moral, room to alter migration flows, emigration cannot be controlled easily. That leaves immigration, which many societies have used to alleviate their problems. It seems likely, however, that in all but a few cases (one of which, interestingly, seems to be Finland) the supply of

potential immigrants is drying up, and where it is not, the potential immigrants are highly undesirable.

This volume therefore gives the impression that national concerns about population are founded upon very uncertain arguments, and that even where action is considered desirable, it is rarely possible to a decisive extent. The interest, perhaps, for scientists is that so few countries pay any attention to the environmental consequences of demographic patterns. In my opinion, that attitude is correct: the connection between population and the environment is at best an indirect one; accordingly, direct measures to protect or improve the environment need be little influenced by population.

G. P. Hawthorn

Man and fire

Fire and Ecosystems. (Physiological Ecology: A Series of Monographs, Texts and Treatises.) Edited by T. T. Kozlowski and C. E. Ahlgren. Pp. xii+542. (Academic: New York and London, December 1974.) \$39.50; £18.95.

To the wildlife conservationist, fire is at the same time a valuable tool and a feared enemy, and it is this love-hate relationship which man has towards fire which forms the underlying theme of this book. It is a collection of regional essays by many eminent ecologists, most of whom are directly concerned with the practical side of forestry, range management or agriculture, and it provides a very extensive review of a subject, which demands the attention of all who are interested in the relationship between man and his environment.

Following a brief introduction there are three chapters, by Viro, Ahlgren and Bendell, of a general nature, which concern the influence of fire on soil, soil organisms, and birds and mammals, respectively. Of these the first, on soil, is very much more limited in its coverage than one might have hoped. Almost all the data quoted concerns podzols in boreal forest areas of Fennoscandia (90% of the literature quoted in the bibliography for this chapter is Scandinavian or Finnish). Although much has been contributed to our knowledge of this subject by scientists from that part of the globe, this presentation represents a very narrow approach to what purports to be a general survey of a very important aspect of the ecology of fire. The chapters on soil microbes and, particularly, on birds and mammals are, however, very full and serve as useful reviews.

There follow, in the main body of the book, sections dealing with the influence of fire on the major biomes of the world, grassland, deciduous and coniferous forests, chaparral and Mediterranean vegetation, and savanna and desert.

Apart from the chapter by Vogl on grasslands, these sections each tend to be devoted to specific geographical regions: for example, the section on forests is divided into four chapters, each dealing with one of the major forested areas of the United States. Although this system of subdivision has undoubtedly made it easier for each of the authors to concentrate on one area of the extensive literature and has also facilitated the task of editing, I feel that some potential has been lost as a result. It would have been useful if some chapters dealing with more general themes could have been incorporated, after the fashion of the first four chapters. But perhaps this attitude is inevitable in west European ecologists as their region is so markedly neglected. Given such a geographically subdivided approach, however, it is sad that space was not found for a chapter dealing with the influence of fire upon heathland and moorland ecology, a subject concerning which there is a wealth of literature. In fact, it is touched upon only briefly, for example, in Bendell's chapter where two pages are given over to the red grouse.

The final chapter, by Kayll, on the use of fire in land management, attempts the kind of synthesis which could have been profitably applied to various other topics in the book. This chapter deserved more space.

Overall, one is left with a book which covers an enormous literature and which is rich in specific examples of fire-adapted species and ecosystems from several parts of the world, but mainly from North America. North American conservationists seeking such specific information will undoubtedly welcome the arrangement of text. Those who seek more general information, however, perhaps relating to fire temperatures and influences upon succession or nutrient cycling processes, will have to collate the numerous, brief references scattered through the text. There are, however, two pervading features for which credit is presumably due to the editors: first, the emphasis upon historic and prehistoric use of fire by man in various biomes and, second, the present day value of fire as a tool in habitat management. These themes are easily traced through most of the chapters, the former reaching a splendid climax in Naveh's biblical and classical account of the history of the use of fire in the Mediterranean region.

The escalating price of this series of monographs on physiological ecology must be remarked upon. This is particularly unfortunate as it is likely to remove them from a rich potential market among nonspecialists, ecologists and conservationists, in whose hands several volumes in the series would have proved particularly valuable.

Peter D. Moore

Oceanology, meteorology and climatology

Oceanology, vol. 1. (Geophysics Series.) Edited by A. P. Kapitsa and P. S. Lineykin. Pp. 127. (Hall: Boston, Massachusetts, January 1975.) \$19.00.

Meteorology and Climatology, vol. 1. (Geophysics Series.) Edited by I. P. Danilina and A. P. Kapitsa. Pp. i+224. (Hall: Boston, Massachusetts, January 1975.) \$29.00.

THESE two books are the first volumes of a proposed series of reviews of recent scientific achievement in geophysical subjects: oceanology, meteorology and climatology, physics of the earth, glaciology, and geomagnetism and the upper atmosphere. Published by the Russian All-Union Institute for Scientific and Technical Information (VINITI) they continue the Summaries of Scientific Progress which first appeared in 1964. Chapters are contributed by Russian authorities in the relevant fields and outline briefly, with some comment, advances made in the two or three years before the original publication in Russian; the volumes thus refer to literature published in the late 1960s and early 1970s. Although the emphasis is placed on Russian work, there is also discussion of research completed in other countries.

The volume entitled *Meteorology and Climatology* includes chapters on physical, satellite and agricultural meteorology, climate and climatic change, photochemistry and the ozone-sphere, and glaciers. The *Oceanology* volume covers sea-atmosphere interaction, sea ice, sea straits, sea forecasts and oceanographic technology. This choice of topics seems somewhat arbitrary, but will probably vary in future volumes. The presentation ranges between the extremes of complete specification of the then current state of knowledge in the particular field and terse chronological summaries of recently published literature, with little reference to earlier research.

Although these works provide insight into the development of various aspects of the sciences, the publication in English some years after their first appearance in Russian rather dates the material discussed. It is felt that researchers in these fields will already be familiar with literature published, even in Russian, five years ago and that their primary interest will be historical.

But value of future volumes in the series would be greatly enhanced if a shorter delay between Russian and English publication could be achieved.

P. M. Kelly

Iron in organisms

Iron in Biochemistry and Medicine. Edited by A. Jacobs and M. Worwood. Pp. xiv+769. (Academic: London and New York, October 1974.) £15.20; \$39.25.

IRON is one of the trace elements present in mammals. It is a transition metal and as such it exists in several oxidation states and forms many metallo-protein complexes. The fascinating multiplicity of structure and function in iron compounds has been investigated by many research workers in widely separated disciplines and their efforts to elucidate various aspects of iron metabolism have yielded a vast amount of valuable information. It has become difficult to keep track of all the published data in several rapidly expanding areas of iron metabolism, but the excellent collection of reviews in *Iron in Biochemistry and Medicine* now makes this task much easier.

In this book 35 active investigators have reviewed the present state of knowledge in their particular areas of interest for the benefit of their colleagues in the same field and for those working in other disciplines. The book comprises twenty chapters, each containing a review of one topic. The topics selected all concern major aspects of iron metabolism.

The biochemistry of iron compounds is discussed in the first half of the book: the structure and function of inorganic iron, transferrin, ferritin, haemosiderin, haem, haem-proteins and non-haem iron proteins are all reviewed in great detail. The chapter on the relationship between iron and other trace metals completes the biochemical section. This is followed by reviews on the physiology and pathology of iron metabolism: iron absorption, iron deficiency and its manifestations and effects, its epidemiology and treatment, iron metabolism in infancy and childhood, the iron and the reticuloendothelial system, and iron overload. Finally, in the last three chapters, the kinetics of iron metabolism, the relationship between iron and infection, and genetic abnormalities of iron metabolism in mice are reviewed.

The book is uniformly well written, with surprisingly little repetition, a tribute to the editorial policy. Many tables and a modest number of illustrations, all thoughtfully prepared, are of great help to the reader, and the references at the end of each chapter are extensive though not comprehensive.

It is perhaps the aggregation of data from widely separated subjects which makes this book so valuable. I would certainly like to have it to hand if I needed to quote the data on iron metabolism from outside the area of my personal interest.

I strongly recommend this book as useful reading for scientists working in

this field. The clinician will find a wealth of biochemical information on the iron proteins he is trying to study by simple means in his patients. The biochemist will learn a lot about the significance of abnormalities of iron metabolism found in diseases. And I am sure that both will obtain considerable help in planning and executing future research.

B. Brozović

Hadron physics

High Energy Hadron Physics. By Martin L. Perl. Pp. xviii+562. (Wiley-Interscience: New York and London, December 1974.) £12.00.

THE nature of the strong nuclear (hadronic) interaction has been the subject of extensive theoretical and experimental investigation over the past 20 years and more, and from time to time books have appeared on aspects of this field, usually impressing upon the reader the need for further understanding of the theoretical complexities involved. This monograph by Professor Perl, an international authority in the field, helps considerably in that respect, and will be of interest and value to postgraduate students and workers at the postdoctoral level. The completion of this book, a *tour de force*, clearly required considerable stamina besides intricate academic knowledge. The author moves along with obvious energy and enthusiasm from the outset to the end, some 500 pages later, generating *en route* considerable respect for both Perl himself and his subject. Were anyone to doubt the usefulness and value of sabbatical leave, let him obtain a copy and read this book. Without such support, most practising experimental physicists would simply not have the time to create work of this quality and detail.

The standard of the book is such that the reader needs a knowledge of the basic theoretical and mathematical background material in elementary particle physics, such as one may acquire from lectures at the first year postgraduate level. Although the style of writing is often conversational, the book demands serious concentration; the considerable formal mathematical manipulation in some chapters makes the going heavy and will demand the continued perseverance of the student. I feel that the low energy nuclear or astrophysicist, who picks up this book and sees from the fly leaf that it will be an "invaluable reference" to him, could well be in deep water very soon.

The book, which contains a reasonably balanced mixture of experimental data and theory, may seem expensive but at present standards it is not excessively so. I hope, and expect, to find the book retained by science libraries for reference.

W. Galbraith

Topics in bioinorganic chemistry

Techniques and Topics in Bioinorganic Chemistry. (Aspects of Inorganic Chemistry.) Edited by C. A. McAuliffe. Pp. xv+351. (Macmillan: London and Basingstoke; February 1975.) £20.00.

THE frontier between inorganic chemistry and biochemistry has been crossed so often that the border is now ill-defined. It is too early to know how the buffer state, which bioinorganic chemistry or inorganic biochemistry may be considered to constitute, will evolve. Certainly, the subject is fashionable. But that which is fashionable is not necessarily respectable, the latter description, evocative of safe, solid virtues, being more often applied to the mainstreams of the parent subjects. But irrespective of prejudices it is impossible to ignore the importance of those elements which normally constitute the province of inorganic chemists, to biological processes. The roles of proteins containing iron and copper in all manners of redox reactions and oxygen transport are well known, and the extent to which other ions, such as zinc, are involved is slowly being revealed. Many other elements, for example, selenium, are known or suspected to be required but their functions ill-defined.

This book is one of several which have appeared recently and which seek to attract attention to the current excitement for the subject. In the first chapter M. W. Makinen attempts ambitiously to relate the functions of some proteins to structural details derived from X-ray diffraction studies occasionally reinforced by information gleaned from spectroscopic investigations. J. M. Pratt, in a chapter entitled "Principles of Catalysis by Metalloenzymes", considers the haemoglobins and myoglobins, catalase and peroxidase, and nitrogenase. The biochemical function of molybdenum is described by F. L. Bowden in a well planned review. Since the environments of molybdenum in proteins such as xanthine oxidase and nitrogenase are unknown, speculation about the role of molybdenum in these proteins is subdued. Some interesting chemistry of molybdenum is, however, included; the relevance to the biochemical function is not overstated. J. Webb discusses a number of complexes and proteins involved in iron transport and storage, with considerable emphasis on the electronic structures of the interacting iron ions. The volume is completed by an all-too-brief introduction by S. J. Ferguson to the use of nuclear magnetic resonance

spectroscopy in the investigation of the structures of proteins in solution.

Books containing chapters by different authors often suffer from two defects. First, the delay between completion of the manuscripts and publication leads, in a rapidly developing subject, to redundant speculation. Second, although the editor in his introduction seeks to justify overlap between the contents of the various chapters, this may prove irritating to those prepared to read through the book. For example, the binding of molecular oxygen to iron in myoglobin and haemoglobin is considered at length in chapters 1 and 2, as is nitrogen fixation in chapters 2 and 3. But as it is likely that the volume will be used as a source of review articles, this criticism may not be pertinent. **H. A. O. Hill**



Eucalyptus jucunda C. A. Gardner, usually 2–3.5 m, but sometimes up to 8 m high. Photo shows stamens and elliptical fruiting bodies (1.5 × 1.2 cm). Taken from *Eucalypts of the Western Australia Goldfields* by G. M. Chippendale. Pp. iv+216. (Australian Government Publishing Service: Canberra, 1973.) AS\$3.75.

Casting light on structures

Biochemical Spectroscopy. By Richard Alan Morton. Vol. 1: pp. xvi+1–381; vol. 2: pp. 382–873. (Adam Hilger: London, March 1975.) £80.00 for set.

THE title *Biochemical Spectroscopy* may today conjure up an image of a work dealing fairly exhaustively with the practical and theoretical aspects of the spectroscopy, primarily, of current molecular biology. It might, for instance, emphasise the applications in that field of the more modern techniques of ESR, Mössbauer and NMR, and perhaps give as much weight to

circular dichroism, optical rotatory dispersion and Raman as to the older basic techniques.

This book is, however, of a quite different kind. Essentially, it consists of a large number of monographs, grouped rather arbitrarily into longer sections. Of these, some, like those entitled 'Water and Sunlight in a Biological Context' and 'Electronic Absorption Spectra', give background material of a relatively generalised kind but most of them are groups of monographs, many quite short, on classes of natural products and the light that spectroscopic methods have cast (and in a few cases might yet cast) on their structures, functions and interrelations. The range of topics is wide, and it is revealing to see in how many and diverse fields the author has himself made contributions in a laboratory career lasting 50 years. A chemist with no special interest in spectroscopy could do worse than to turn first to this book if he should have to deal with a natural product belonging to a known but unfamiliar class; the treatment of the chemistry is always interesting, if necessarily incomplete, and the references are excellent. The writing is clear and straightforward, and, though the bias is towards classical visible and ultraviolet spectroscopy, developments based on other techniques are not neglected.

In places the reader encounters unexpected hurdles, however. Some of these arise from a failure to number, and thus to correlate with the text, the numerous structural formulae embedded in it, and in places the lack of either names or reference numbers, coupled with a sprinkling of errors, makes the going hard indeed for a reader unfamiliar with the field. The sources of much of the tabulated data are not given, and some sections wear the antique look which is conveyed by the bulgy contours of 30 or 40 year-old spectral diagrams; these, however, normally occur where the more modern data which might otherwise supersede them are incomplete. Although in places some updating of material is surely needed, a prime defect is the plethora of small errors and misprints which defaces this handsomely produced—and extremely expensive—pair of volumes.

The price is such, in fact, that the volumes can only be considered as a library reference work. This is a great pity, since a deal of pleasure and information is to be had from random browsing. It is, however, clearly impossible to suggest cuts and economies in presentation which could result in a price reduction by the order of magnitude which is needed to make the volumes as accessible as they deserve to be. **E. A. Johnson**

announcements

Award

The **Kelvin Gold Medal** has been awarded to **C. S. Draper** for his work on the problems of guidance and control in space.

Appointments

R. C. Newman has been appointed Professor of Physics at Reading University.

D. Givol has been named the first incumbent of the Altschuler Chair of Immunochemistry.

J. D. Gillett has been appointed the first Pro Vice-Chancellor of Brunel University.

Miscellaneous

Culture Collections. The United Kingdom Federation for Culture Collections, designed to assist microbiologists, is now in existence. Further information can be obtained from Dr I. J. Bonsfield, Torry Research Station, PO Box 31, 135 Abbey Road, Aberdeen, Scotland.

International meetings

September 29–October 1, **Biological implications of metals in the environment**, Richland, Washington (Miss J. H. Rising, Biology Department, 331 Building, Battelle-Northwest, Richland, WA99352).

October 7–9, **Techniques for the microbiological examination of foods, pharmaceutical and cosmetic products**, London (Scientific Symposia Ltd., 121 King Street, London W6 9JG, UK).

October 20–24, **Clean air and pollution control**, Brighton (The Secretary, National Society for Clean Air, 136 North Street, Brighton BN1 1RG, UK).

October 29–November 1, **Chemical evolution of the early precambrian**, Maryland, US (Dr Cyril Ponnamperna, Laboratory of Chemical Evolution, Department of Chemistry, University of Maryland, College Park, Maryland 20742).

October 25–29, **Medical radionuclide imaging**, Los Angeles (The Secretary, International Atomic Energy Agency, PO Box 590, A-1011 Vienna, Austria).

October 27–31, **Heavy metals in the environment**, Toronto (Mr K. Ward, Executive Secretary, National Research Council of Canada, Ottawa, Canada K1A 0R6).

Person to Person

Printing of mathematics. Could anyone loan or sell me a copy of *The Printing of Mathematics* (Oxford, out of print)? Also any other books on mathematical printing. Will pay postage. Vic Cresswell, Dawkins Typesetters, Graphic House, Southwark Street, London SE1 or (01)-407 7525.

Seeds and latex. Investigator will appreciate knowing where to obtain seeds and latex (1–2 kg of each) of *Hura crepitans* (*Hura brasiliensis*) (Professor F. Stirpe, Istituto di Patologia generale, Via S. Giacomo 14, 40126 Bologna, Italy).

Endoplasmic reticulum. Biologists writing a book on the structure of living cells would be grateful to know of any publications which have indicated that the endoplasmic reticulum might be an artefact. (Dr H. Hillman and Mr P. Sartory, Unity Laboratory, Department of Human Biology, University of Surrey, Guildford, Surrey GU2 5XH).

There will be no charge for this service. Send items (not more than 60 words) to Holly Connell at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

October 30, **Recent chemistry of natural products, including tobacco**, Richmond (Nicholas J. Fina, The Phillip Morris Research Center, PO Box 26853, Richmond, Virginia 23261).

October 30–31, **Comparative biology of skin**, London (Dr R. I. C. Spearman, The Zoological Society, Regent's Park, London NW1 4RY, UK).

Reports and publications

Great Britain

Timber Research and Development Association. Annual Report for 1974. Pp. x+16. (High Wycombe, Bucks: Timber Research and Development Association, 1975.) [27]

1975 Conference on Dielectric Materials, Measurements and Applications, 21–25 July 1975, Churchill College, Cambridge. (Organized by the Science, Education and Management Division of the Institution of Electrical Engineers, in association with the Institute of Electrical and Electronics Engineers (United Kingdom and Republic of Ireland Section); the Institute of Physics; and the Institution of Electronic and Radio Engineers.) Pp. xii+336. (London: The Institution of Electrical Engineers, 1975.) [27]

Explore the New Forest. Edited by Donn Small. (An official guide by the Forestry Commission.) Pp. 125. (London: HMSO, 1975.) £1.85 net. [37]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 278, No. 1286: The Influence of Pore-Water Tension on the Strength of Clay. By A. W. Bishop, N. K. Kumapley and A. El-Ruwaihi. Pp. 511–554 + Plate 17. UK £3.95; Overseas £5.10. Vol. 278, No. 1287: The Initial-Value Problem for the Korteweg-de Vries Equation. By J. L. Bona and R. Smith. Pp. 555–604. UK £1.95; Overseas £2. (London: The Royal Society, 1975.) [37]

The Lister Institute of Preventive Medicine. Report of the Governing Body, 1975. Pp. 22. (London: The Lister Institute of Preventive Medicine, Chelsea Bridge Road, SW1, 1975.) [47]

Association of the British Pharmaceutical Industry. Annual Report, 1974/75. Pp. 44. (London: Association of the British Pharmaceutical Industry, 162 Regent Street, 1975.) [77]

Potato Marketing Board. Machinery Directory. Pp. 44. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1975.) 15p. [87]

Rowett Research Institute. Annual Report of Studies in Animal Nutrition and Allied Sciences, Vol. 30, 1974. Pp. 143. (Bucksburn, Aberdeen: Rowett Research Institute, 1975.) £1; \$2.30. [87]

National Vegetable Research Station. 25th Annual Report 1974. Pp. 166. (Wellesbourne, Warwick: The British Society for the Promotion of Vegetable Research, National Vegetable Research Station, 1975.) £1.25. [107]

Bulletin of the British Museum (Natural History). Geology, Vol. 26, No. 2: The Shell Structure of the Liassic Ammonite Family Dactyloceratidae. By M. K. Howarth. Pp. 45–67 + 10 plates. (London: British Museum (Natural History), 1975.) £2.70. [107]

Proceedings of the Royal Irish Academy, Vol. 75, Section A, No. 10: Luminescence from Chromium Doped Yttrium Aluminium Garnet. By M. O. Henry, J. P. Larkin and G. F. Imbisch. Pp. 97–106. 35p. Vol. 75, Section B, No. 15: The Distribution and Morphometried of Spittle Bugs on Irish Blanket Bog. By N. Nixon, Elaine F. Okely and Ruth M. Blackith. Pp. 305–315. 29p. (Dublin: Royal Irish Academy, 1975.) [107]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 271, No. 911: A Discussion on Forests and Forestry in Britain. Organized by G. D. Holmes, P. F. Wareing and J. L. Harley. Pp. 45–232. (London: The Royal Society, 1975.) UK £7.75; Overseas £7.95. [107]

European Spectroscopy News, Vol. 1, No. 1, 1975. Pp. 1–32. Edited by P. M. Williams. Published Bimonthly from July 1975. (London: Heyden and Son, Ltd., Spectrum House, Alderton Crescent, NW4, 1975.) Free to all Practising Spectroscopists. [107]

London and Home Counties Regional Advisory Council for Technological Education. Science Education in the Region, 1975/76. Pp. 55. (London: London and Home Counties Regional Advisory Council for Technological Education, 1975.) 70p. [147]

Responsibilities of Industry in the Field of Health. By Dr. J. H. Briggs and Dr. Robert Murray. (Foundation Dialogues.) Pp. 16. (London: Foundation for Business Responsibilities, Portland House, Stag Place, SW1, 1975.) 25p. [157]

Union Internationale des Laboratoires Independants. Register of Members 1975. Pp. 237. (London: Union Internationale des Laboratoires Independants, Ashbourne House, Alberon Gardens, NW11, 1975.) [157]

Freedom and Restraint in Broadcasting: The British Experience. By Sir Michael Swann, FRS. (The 'Queen' Lecture given in the Kongresshalle, Berlin, 29 May 1975.) Pp. 31. (London: BBC, 1975.) [157]

Public Health Laboratory Service. Monograph Series No. 8: Isolation of Salmonellas. By R. W. S. Harvey and T. H. Price. Pp. 52. (London: HMSO, 1974.) £1.50 net. [157]

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nature

September 18, 1975

No easy way to solve universities' problems

IN 1970 the number of students entering universities in the United Kingdom to study dentistry (1,170) was a mere third of the number planning to study chemistry. The 1974 entry was somewhat different, according to the statistical supplement to the Twelfth Report of the Universities Central Council on Admissions (UCCA). There were 3,065 prospective dentists, one and a half times the number of prospective chemists. Provisional figures for 1975 show the trend halted, but no more.

This is the most extreme example. The swing would be somewhat less if, say, medical students and physicists were compared, but the point must surely now have penetrated the consciousness of even the most optimistic or ostrich-like scientists: that a totally unplanned manpower shortage of scientists is creeping up on us.

Ironically, at the same time an equally unplanned shortage of job opportunities within universities has arisen, with the unwillingness of most universities to take on long term staff commitments during the past two years. It would be incorrect to see these two shortfalls balancing each other out and so preventing the worst from happening; only a small fraction of university entrants eventually opt for a university career. The universities are in trouble, and university science is in the most trouble of all.

It is against this depressing backdrop that the House of Commons Select Committee on Science and Technology issues its interim report on the short-term problems of scientific research in British Universities (House of Commons Paper 504, HMSO, 65p). Nothing in the report will be a great surprise; the committee has not fallen for any one witness nor has it advanced any patent remedy of its own. Instead there is a sober assessment of the situation and some muted pleas to the Department of Education and Science and the University Grants Committee to hold the line on the balance between teaching and research in universities and to the universities to look carefully at their individual priorities.

Research in universities looks a precariously marginal activity when viewed from Whitehall. The Department of Education and Science is very dominantly a department of education. The University Grants Committee sees research and educational elements as indistinguishable when it allocates money, but reckons research only to comprise "rather more than 25%" of the totals. The research councils put less than one-third of their money into university departments by way of grants, studentships and fellowships. In every way university research is vulnerable to the stirrings of its larger administrative

bedfellows. Some of those bedfellows, such as academic salaries, not only rightly take precedence, but also may be committed for the next 10, 20 or more years.

And yet a crisis need not be a bad thing, particularly in an environment which has known nothing but growth for years. The committee itself remarks that if inflation concentrates university minds, it may be of some benefit. The opportunity to review priorities and seek the most efficient means of deploying more limited funds is one which it would be "highly regrettable" for the universities to ignore. Linked to this broad hint is a similar one that universities must review the relevance of their research activities. This statement is a bit of a compromise; there was at one time within the committee a feeling that there should be a national body—perhaps a 'de-grants committee'—to perform this function. And it is made clear that this idea is only temporarily on ice.

Can and should the universities respond to such a challenge to assess their priorities both in general and in terms of relevance? The answer is almost certainly that individually they lack the tools to do so or indeed much perception of how to assess relevance. The vice-like grip of job security so severely restricts the universities' freedom of action that opportunities for change, redeployment or tapering off in the staffing of departments is restricted, even in good times, largely to the arbitrariness of death, resignation or retirement. Certain economies are no doubt still possible by sharing of facilities and so on, but when 80% or more of the universities' general funds are committed to salaries and wages it is clear that the scope is limited if personnel levels are maintained.

Even more difficult to respond to is the challenge that universities must review relevance. First, there would certainly be sniping across the great divide between arts and sciences; is mediocre electrical engineering less or more relevant than competent Old Testament theology? Second, some of the relevancies can only be seen in a national perspective; maybe mathematical geodesy or Amharic look pretty irrelevant in a local context, but maybe there is a national need for a few graduates a year. Third, demands for relevance could lead to the sight of academics presenting unedifying inflated and misleading claims for their work rather in the way that confessions are 'volunteered' in some countries.

The committee rightly identifies the principal crisis as one of confidence in the future. A veiled threat to review relevance before the committee does it for you will hardly bolster this confidence. □

THE POOR CLIMBING BOYS.

A HUMANE British Parliament passed an Act several years ago, known as Lord Shaftesbury's "CLIMBING BOYS' ACT," whereby the barbarous employment of poor children for sweeping chimneys was prohibited, and the use of machines enforced. So far as London is concerned, this Act is, we believe, faithfully enforced,—but in the provinces there are at the present time upwards of 3000 of these poor creatures still suffering the wrongs of British slavery.

As the bulk of the climbing boys come from the families of working-men, who by accident, or the premature death of their parents, are thrown into Union Houses—we appeal to our readers to do what they can to enforce the faithful carrying out of the Act of Parliament. Mr. Wm. Wood, of Bowden, Manchester, or Mr. James Glass, of 24, Barrington Crescent, Brix-



"PITY THE POOR CLIMBING BOYS!"

ton, will gladly correspond with any parties who desire to aid in preventing the use of climbing boys in districts where they are at present employed.

Cancer at work

Although there has been progress in the treatment of cancer by radiation, surgery, chemotherapy and immunotherapy, it is probable that efforts to prevent the disease have been more rewarding and effective than those made to improve treatment. Some occupational cancers can be prevented when the cause is identified. It is, however, often difficult to find the causes and sometimes even more difficult to remove them when they are known. Two hundred years after Percival Pott made the connection between cancer and the working environment, Professor E. Boyland reviews subsequent work on occupational cancers.

PERCIVAL POTT, FRS, was a surgeon at St Bartholomew's Hospital; his "Chirurgical observations relative to the cataract, the polypus of the nose, the cancer of the scrotum, the different kinds of ruptures and the modification of the toes and feet" was published by Hawes and Collins in Pater-noster Row in 1775. The paper entitled "cancer scroti" describes "a disease as peculiar to a certain set of people which has not, at least to my knowledge, been publicly noticed; I mean the chimney-sweepers' cancer". "The trade call it soot-wart. The disease, in these people, seems to derive its origin from the lodgement of soot in the rugae of the scrotum". He had identified a cause of cancer—a carcinogen active in man. The observations and identification did not stop the exposure of the child chimney sweeps, and Charles Kingsley, who was an active member of the Christian Socialist movement led by Frederick Denison Maurice, described the plights of these child workers in *Waterbabies* almost a century later.

Pott pointed out that "the subjects are young, in general in good health, at least at first, the disease brought on them by their occupation—all this makes it (at first) a very different case from cancer which appears in an elderly man, whose fluids become acrimonious from time, as well as other causes; or from the same kind of complaint in women who have ceased to menstruate. But be all this as it may, the scrotum is no vital organ, nor can the loss of a part of it ever be attended with . . . the smallest degree of inconvenience". He must have perceived the process which is chemical carcinogenesis.

Percival Pott was a modest scholar; as his son-in-law, Sir James Earle, wrote "he often said he began to teach when he had much to learn; and that he was not actuated by that opinionative wisdom which sometimes attends advanced life, after all his study and experience he confessed that he still retained a long list of inquirenda". Samuel Johnson was one of his patients and his portrait painted by Joshua Reynolds hangs in St Bartholomew's Hospital. His epitaph reads "The labours of the ancients were familiar to him; he scorned to teach a science of which he had not traced the growth."

The Romans had chimneys in connection with their hot air heating systems, but in northern Europe chimneys were developed slowly. In mediaeval times hearths in the centre of the living room and vents in the roof were usual. When coal became available as a domestic fuel Count Rumford established the forms and proper relationships of the parts of the chimney. The

chimneys which required children to clean them were an English development and the associated disease was more common in England than in continental Europe. It seems probable that chimney sweeps in Germany had some form of protective clothing.

Before Percival Pott, Ramazzini in the *de morbis artificum*, published in 1700, considered occupational diseases such as the colic due to lead to which painters, glaziers and plumbers were exposed. Ramazzini had noticed the high incidence of breast cancer in nuns and indicated that this was caused by their occupation.

Following the work of Pott many clinicians reported on the condition. Bell in 1794 described other cases and said it appears obviously to be produced by soot, for it is found that besides chimney sweeps those who are employed in manufacture in which soot enters are occasionally seized by it. James Earle in 1808 recorded a case of a gardener with epithelioma of the left hand on which he had previously hung a pot containing soot used to kill slugs. He saw that skin cancer elsewhere than in the scrotum could be caused by soot. In the edition of the works of Percival Pott, revised by James Earle in 1808, reference was made to an eight-year-old apprentice with scrotal cancer. Often, however, there was a long latent period. Curling in 1856 described a case of a sailor who developed scrotal cancer in the fifth decade of life but who had been brought up as a sweep. In the nineteenth century the disease remained but it was recognised that not all children employed as sweeps developed scrotal cancer.

With the expansion of the Industrial Revolution increasing amounts of oils were used for the lubrication of machines. Before 1850 the oils were mainly of animal origin but later mineral oils were used in much larger quantities. Volkmann in 1875 described occupational skin cancer by tar, paraffin and soot in Germany and in the following year (1876) Bell of Edinburgh described cases of "paraffin cancer" in workers in the Scottish shale oil industry.

As the incidence of chimney sweeps' cancer decreased in the nineteenth century the incidences of scrotal cancer increased among the spinners of the Lancashire cotton industry, the first cases being described in 1887. This "mule spinners' cancer" has decreased in the present century because (1) the cotton industry employs fewer workers, (2) ring spinning machines have replaced the old mule spinners, (3) less carcinogenic oils controlled by specifications are used, and (4) there has been a general improvement in hygiene. Although less common, scrotal cancer

still occurs in industry in conditions in which men are exposed to lubricating oils.

Although the first synthetic dyestuffs were made in England by Perkin, the German chemical industry was the first to exploit the discoveries and manufacture dyestuffs on a large scale. It was in Germany that the first cases of bladder cancer due to exposure to aromatic amines were reported by Rehn in 1895. The development of dyestuffs industries in other countries was associated with widespread increases in the incidence of bladder cancer. Cases of bladder cancer in men who had manufactured dyestuffs intermediates including 2-naphthylamine and benzidine in England were reported by Wignall in 1929. Another aromatic amine, 4-aminobiphenyl, had been manufactured and used in the United States for many years. The intelligent anticipation and laboratory work of Williams and his colleagues (1952), showing that it was carcinogenic to animals, prevented the manufacture of this compound in Britain and other countries. In 1955 evidence was presented showing that this compound had caused cancer in men occupied with its manufacture.

In addition to their use in dyestuff manufacture, aromatic amines were used in the rubber industry. Case and Hosker in 1954 found an increased



Pott by Reynolds (courtesy Bart's Hospital)

incidence of bladder cancer in rubber workers in one region of England. It is probable that carcinogenic aromatic amines have not been used in the rubber industry since 1950 and because of the possible hazard special facilities for early diagnosis have been available for 20 years. Cancer of the bladder has been a prescribed disease in Britain since 1953.

Early in the present century Japanese workers demonstrated that the application of coal tar to the ears of rabbits or the skin of mice induced tumours some of which were malignant. This provides a bioassay of carcinogenic activity which Kennaway and his colleagues used to identify the hydrocarbon 1, 2,5, 6-dibenzanthracene as the first known compound to induce cancer and then for the isolation and characterisation of 3, 4-benzopyrene from coal tar. Carcinogenic aromatic amines and polycyclic hydrocarbons were known 40 years ago, but in spite of much research the mechanisms by which they exert their carcinogenic actions are still not exactly known.

The knowledge of the chemical nature of the carcinogens, which must have been present in the soot that caused cancer in the young chimney sweeps described by Percival Pott, has in some cases helped with the problems of reducing the hazard of environmental carcinogens. Many other extrinsic cancer-producing agents have been discovered. As most cancers in man are probably caused by extrinsic factors, many still remain to be identified. The discovery of these and the removal or reduction of them in the environment will decrease the incidence of cancer in the way started by the English surgeon two hundred years ago. □

THE young scientist who wants to venture into medical research now faces an acute problem in finding a satisfactory career structure. It used to be that workers in this field had some form of tenure, either through their contract or through a moral obligation readily acknowledged by the employer. But the trend in recent years has been to offer only temporary appointments lasting, at most, five years. This poses severe problems.

The really outstanding person will always find help elsewhere if the support runs out, of course. My concern is more for the capable and well qualified scientist. Three to five years of medical research may actually prove to be a handicap when competing for teaching or research posts with candidates who have pursued a more academic path.

The Medical Research Council have recently recognised the problem in their own establishments by ensuring that tenure will be granted to staff no later than in their mid-30s—other institutions will probably fall into line. But this only accounts for 20–30 people annually. Numerous first-rate scientists better equipped for work in applied medical research are still un-

helped by this scheme.

In the UK, experience has shown that the best way to effect the transition from research activity to clinical trials is through a team of medical and scientific staff who can meet

Career medicine

regularly on an equal footing. (Contrast this with the United States where young doctors may undergo a long training in basic science and later become competent to direct highly advanced work in a hospital laboratory.) Such teams at present exist in advanced institutes and in them the scientist (who often makes the early running) has learnt to communicate successfully with the clinician. Might not such teams be introduced more widely in medical schools and general hospitals? They could be a highly efficient means of speeding the acceptance by clinicians of the most recent advances in laboratory methods. And might not this be a solution to the career problem for medical scientists?

Such a scheme could best start in specialised centres or medical schools where there already is a tradition of regular clinical conferences. Young medical staff would rapidly recognise the advantages of team work with scientists; it is all but impossible to keep pace with advanced laboratory methods as well as advanced medicine.

Recruitment of scientists to such a service could take place in two ways. Firstly, at the graduate level, for which the Universities might set up an M.Sc. course in Bio-Medical Science as a recognised qualification. Secondly, scientists who have already spent three to five years in medical research might be required to take a diploma in Bio-Medical Science. These courses would provide a general clinical background to the problems in which the hospital laboratories play a major role a large proportion of the lectures being given by medical staff. This would be far less expensive for the country than the training of medical graduates in advanced laboratory methods, in addition to their medical training. Such a scheme could provide a career structure for those scientists at present in medical research who have a most uncertain future.—E. J. Ambrose.

international news

As Britain prepares to face another winter with further rises in the price of fuel, the Department of Energy, formed less than two years ago to deal with the problems of energy crisis, has become the target of heavy criticism in a report prepared by the House of Commons Select Committee on Science and Technology (House of Commons Paper No. 487, HMSO, 1975). Among the proposals contained in the report is a call for new administrative machinery at Ministerial level to co-ordinate a policy intended to cut Britain's annual energy bill by £1,000 million (about 15%).

According to the chairman of the all-party committee, Mr Arthur Palmer, a reorganisation is needed because of the failure of the present energy administration to implement policies of energy conservation effectively. He said last week that very little had been done during the past year to improve energy efficiency and that the committee did not share the government view that the recent diminution in energy demand, largely attributable to the recession, represented a genuine saving. The problem of energy conservation, he said, had not been considered with sufficient urgency by the existing administration, apparently because of the tendency in government circles both to treat issues requiring practical and administrative attention in an abstract and political manner, and to consider important long term issues only in the light of their short term political implications.

In all, the report lists 42 recommendations for increasing efficiency in the use and supply of energy. Underlying the proposals is a recommendation that a full time "task force" of Ministers, officials and outside experts be set up to meet the problems of the continuing energy crisis. If the government decides to act on the committee's proposals after the report has been considered by Parliament, the new administration would replace the Advisory Council on Energy Conservation (ACEC) and the Advisory Council on Research and Development (ACORD) both of which are responsible to the Department of Energy at present. Nonetheless, that would not mean the demise of the Department of Energy itself, which would remain responsible for the more specific problems of coal, offshore oil and gas and nuclear power. Indeed, the report notes

British energy savers taken to task

by Allan Piper

that it may be the department's involvement with those matters that has led to what is seen as a lack of central direction and decisiveness in matters of conservation.

If it set up, the main function of the task force will be to ensure a greater efficiency of energy use in every area of the economy. Operating on its own budget, it will work within a time limit towards clearly defined objectives. On the basis of the information collected from expert witnesses interviewed during the preparation of the report, the committee suggests that a realistic target is a reduction in energy consumption of 15% within three years. That could be achieved, according to the report, "without sacrificing output, employment, or living standards". Additionally, the report further points out that an effectively directed programme of energy conservation could be sustained at a lower cost than the development of an equivalent amount of additional energy from North Sea resources.

The effectiveness of the task force will, it is hoped, rest mainly in its regional structure. Recognising the need for a more direct implementation of policies than there has been in the past, the committee believes that future policies can only be successful if the task force establishes a strong local organisation.

Exactly how the task force will fit in with the Department of Energy is not entirely clear, however, though the committee recommends that it should be set up at Ministerial level and should report directly to the Prime Minister. Questioned on the purpose of establishing another administrative body in addition to the Department of Energy, Mr Palmer said the committee felt it was necessary because the problems of energy conservation cut across existing departmental boundaries. As an example, he cited the Department of Industry and the Ministry of Defence.

Both, he said, are in need of effective policies of energy conservation, but neither falls within the jurisdiction of the Department of Energy. The new administration, on the other hand, would possess sufficient political status to implement effective policies wherever necessary.

On the industrial front the report calls for the introduction of a new grants scheme that should be particularly favourable to smaller interests and is designed to encourage the introduction of more efficient plant. Industry accounts for about 40% of British energy consumption, and the wastage is particularly high. It therefore seems likely that the committee's call for legislation to cover the industrial use of fuels and the running of industrial equipment will receive serious consideration. Furthermore, the benefits to be gained by using the experience of independent consultants on energy-saving schemes is recognised in the report, and the committee suggests that they could be suitably employed as agents by the task force. In the committee's view the National Industrial Fuel Efficiency Service (NIFES), the largest consultant organisation in the field, with 136 engineers, would be particularly well equipped to cope and could, even, be reintroduced advantageously to the public sector.

The committee has listed several recommendations for preventing domestic energy wastage and profligate use of energy in the areas of public and private transport. The report suggests that new buildings should be constructed to a specific standard of insulation, with legislation introduced to ensure the implementation of minimum requirements within the home. There is also a call for the maintenance of advertising campaigns to impress on the public the need for a greater awareness of the continuing energy crisis. In that respect the Department of Energy is criticised for its past record and is accused of following a policy "feeble in contrast to the need for strong action". One recommendation that seems long overdue is that the energy supply industries—the electricity and gas boards—must reconsider the scheme whereby consumers pay proportionally less for additional consumption beyond a certain point.

On the question of transport the committee makes the interesting suggestion that inland waterways and

As an example of how not to regulate environmental chemicals, the case of diethylstilboestrol (DES) is a tough one to beat. A feed additive which stimulates growth in cattle, DES has been enmeshed in bitter controversy for more than a decade. The problem is that DES is highly carcinogenic, and traces of it can sometimes be found in meat and beef liver. Consequently, after bending over backwards for years in an effort to find a way to keep DES on the market the Food and Drug Administration (FDA) in 1973 issued an order banning its use. But the ban was thrown out in court because the FDA had ineptly failed to follow the correct procedure when it passed sentence. The result is that DES is still on the market and is now found in beef liver at higher concentrations than when it was banned two years ago.

That sorry series of events prompted the Senate last week to pass an unprecedented bill banning the use of DES in cattle feed, at least until the FDA can prove that it is safe. If the House of Representatives follows suit—prospects there are uncertain—it will be the first time that Congress has legislated against a single chemical.

The case against DES is a complicated one, which involves weighing the potential risks of long term exposure to extremely low levels of a known carcinogen against the economic benefits of bringing cattle to market more quickly. It is not something at which politicians are particularly adept, and the debate certainly proved that the floor of the Senate is not a particularly good place to reach complex regulatory decisions.

Diethylstilboestrol is one of the few chemicals for which there is good evidence that it causes cancer in man. Recently there have been a number of cases—220 have come to light so far—of an extremely rare vaginal cancer in young women in their late teens and early 20s; a common factor among the women is that their mothers took DES as a drug during the late stages of pregnancy to prevent miscarriage (a practice now discontinued). With such evidence at hand, there is good reason to keep DES out of foods.

But DES is an extraordinarily good growth stimulant for cattle, and it originally seemed that if it was withdrawn from cattle feed a few days before the beasts were slaughtered, it would all be excreted and no residues would contaminate the meat. Things did not work out like that, however, because in spite of FDA regulations

Washington seen

by Colin Norman

requiring cattle to be taken off DES at least a week before slaughter, residues continued to show up in beef and liver.

The levels at which it is present are, however, extremely low, and here the discussion gets into the controversial area of whether or not there is a threshold dose of a carcinogen below which it presents no health hazard. The Senate debate proceeded with those supporting a ban, led by Senator Edward M. Kennedy, citing a sheaf of reports suggesting that known carcinogens should be kept out of the environment completely, while opponents of the ban argued that DES is present in meat at such lower levels that it poses no risk. In the end, the ban was approved by a vote of 61 to 29, and everybody was left wishing that the FDA had been less inept when it first tried to ban DES.

● Acting with a degree of unanimity that is rare in the top echelons of the scientific community, 186 eminent scientists have endorsed a statement condemning astrology as pernicious, anti-scientific nonsense based on magic and superstition. Published in the September/October issue of *The Humanist*, the journal of the American Humanist Association, the statement notes that astrology "pervades modern society" and suggests that "the time has come to challenge directly, and forcefully, the pretentious claims of astrological charlatans".

Proceeding from the observation that "in ancient times people believed in the predictions and advice of astro-

logers because astrology was part and parcel of their magical world view ... [and] they had no concept of the vast distances from the Earth to the planets and stars", the statement notes that "now these distances can and have been calculated, we can see how infinitesimally small are the gravitational and other effects produced by the distant planets and the far more distant stars". Consequently, "It is simply a mistake to imagine that the forces exerted by stars and planets at the moment of birth can in any way shape our futures. Neither is it true that the position of distant heavenly bodies can make certain days or periods more favourable to particular kinds of action, or that the sign under which one was born determines one's compatibility or incompatibility with other people".

The statement was drafted by Bart J. Bok, Emeritus Professor of Astronomy at the University of Arizona, Lawrence Jerome, a science writer, and Paul Kurtz, Professor of Philosophy at the State University of New York at Buffalo. It was sent to about 300 scientists, chiefly astronomers and astrophysicists, during the summer, and about 60% of them responded positively. Among the signatories are 18 Nobel Prizewinners.

Given the eminently sensible and unarguable contents of the statement, it is not surprising that it attracted so much influential support. But why was it deemed necessary to open a frontal assault on astrology at this point in time? Kurtz said last week that he is disturbed by the burgeoning interest in astrology, particularly in the United States (where, according to one estimate, there are some 20,000 practising astrologers), and Bok noted in a separate article in *The Humanist* that some universities and junior colleges even offer courses in astrology. The statement itself also condemned "the continued uncritical dissemination of astrological charts, forecasts and horoscopes by the media and by otherwise reputable newspapers, magazines and book publishers [which] can only contribute to the growth of irrationalism and obscurantism".

coastal shipping routes should receive more extensive use than at present. Perhaps alarming to the private motorist will be the call for consideration of severe restrictions on the use of private transport in one or two selected cities—Mr Palmer mentioned Bristol, his home constituency as a suitable example. Mr Palmer also mentioned the committee's suggestion that companies should establish car pools rather than provide individuals with cars.

As for the less immediate future, the government is urged to consider the use of alternative sources of energy such as nuclear fusion and hydrogen fuel. Significant to that proposal is the recommendation that the Energy Technology Support Unit (ETSU), at Harwell, primarily responsible for research into new energy projects under the leadership of the Department of Energy's Chief Scientist, Dr Walter Marshall, should be expanded and

strengthened by the task force. Mr Palmer also mentioned what he referred to as "way out" sources, and said that in particular the committee had been interested by the idea of tidal power. The Severn Estuary is in fact well suited to provide energy from that source and has already been the subject of several research schemes. Presumably, solar energy and wave power will also come under scrutiny if the committee's proposals are adopted. □

THE right of the government of the host country to an international conference to refuse entry visas to foreign delegates has long been a vexed one. The Tbilisi conference on artificial intelligence (September 2–8) produced an interesting variation on this problem: the right of the government of the host country to refuse to admit its own nationals.

The problem in this case was that of Jewish refusnik scientists, still living within the Soviet Union but dismissed from their academic posts while awaiting a visa for Israel. Although the Soviet scientific hosts of the conference were quite willing to admit them, the KGB was totally opposed to this and considered their wish to attend as being a "provocation". The international organising committee, however, made strong representations that, should these scientists be refused permission to attend, there would be forceful protests and resulting adverse publicity. Consequently, for fear of a withdrawal by the USA and other Western delegations, and as a result of last minute meetings in Moscow, the authorities decided to permit Dr Aleksandr Lerner to travel from Moscow to attend the conference. He was escorted to Tbilisi by the same KGB officer who had formally told him he could not go and welcomed by the head of the Cybernetics Institute of the Georgian Academy of Sciences, Dr V. V. Chavchanidze, who had himself photographed pinning on Lerner's conference badge. Although subjected to a certain amount of official surveillance throughout the conference, Lerner was able to circulate freely among the delegates and take part in

a panel session "Cybernetics, Mathematics and Artificial Intelligence".

Less fortunate were the Gold'shtein brothers, Isai and Grigori. Being residents of Tbilisi, they did not have the initial difficulties of travel permits and hotel registrations; nevertheless, after meeting with newly arrived delegates and talking with them on scientific

Soviet diary

from Vera Rich

topics and also on the difficulties of life as a refusnik, they were called in by the KGB and warned to stay away from the area of the conference building, unless they had had an official invitation from the conference committee. It was not until Saturday, September 6, the penultimate working day of the conference, that Elizaveta Bykova, the wife of Isai and herself a physicist, managed to convey this message to the committee. After a long and forceful meeting between the conference committee and the Georgian hosts, a compromise was reached by which Dr Chavchanidze undertook to invite them for the remainder of the conference on his own personal initiative—a singular example of moral courage.

This reluctance to admit the refusniks seems to be related to the attitude to Israeli scientists. No scientists resident in Israel were allowed to attend, and one Israeli, Dr Y. Yakimovsky, at present working in the USA, received a promise of a visa far too late for him to attend. Nevertheless, two other

Israelis from the USA, Judah Pearl and Meir Weinstein, were permitted to attend, so that the Soviet authorities fulfilled at least the letter of the unofficial working agreement on international conferences—that although individual delegates may be refused entry, no national delegation will be excluded *in toto*.

● The Presidium of the Supreme Soviet of the USSR has announced that "The Order of Friendship of Nations" has been awarded, individually, to each of the Academies of Science of the Union Republics of the USSR, namely, to the Ukrainian, Byelorussian, Uzbek, Kazakh, Georgian, Azeri, Lithuanian, Moldavian, Latvian, Kirgiz, Tadjik, Armenian, Turkmenian, and Estonian Academies, "for service in the development of Soviet science, economics, and culture, and in the training of highly qualified scientific cadres". One Republic only is not so honoured—the Russian Federated Republic, which has no academy of its own.

● Reconstruction work has begun at the highest botanical garden in the world, at Khorog in Tadzhikistan. The garden, at a site in the High Pamirs some 2000 m above sea-level ("above the clouds") is extensively used for field work on the introduction and acclimatisation of plants in extremal conditions. The plants, received on a basis of a regular exchange of seed with 30 Soviet and 36 foreign botanical gardens, therefore include not only and the Hindu Kush, but also more high-altitude flora from the Himalayas plebeian species, including several strains of potatoes, and that mainstay of agricultural planning of the Khrushchev era—maize.

THERE are indications that the new head of the Soviet space establishment, Academician Roald Sagdeyev, has wider interests in space cooperation than costly "spectaculars". Space scientists of the West first made his acquaintance at this summer's COSPAR Symposium in Bulgaria (COSPAR is shorthand for the ICSU Committee for Space Research, the principal international scientific—as opposed to technological—meeting ground). The impression he made was fresh and favourable: he is youngish, full of ideas, and expresses them in excellent English. This is not quite the image projected by his long-surviving predecessor, Academician Blagdonravov—a loveable octogenarian if ever there was one—who died earlier this year.

Sagdeyev's actual post as head of the USSR Institute of Space Research in Moscow puts him in a key position to monitor international developments at

Plans for space

from Angela Croome

least in the physical sciences. Soviet development of ideas for experiments must surely have seemed to stagnate since the 1950s; sputniks were becoming not only relatively smaller but stupider. At the COSPAR meeting, Sagdeyev was not only responsive to the idea of international cooperation in certain fields but showed particular interest in getting involved in X-ray astronomy projects.

On the organisational front, the initiative seems to have passed from the USA (at least temporarily). The formation of the European Space Agency (ESA) as a matching body to NASA has thrown into prominence the lack of a learned body in Europe, comparable with the Space Sciences Board of the US National Academy of Sciences, to give informed but inde-

pendent opinions on the long term objectives of space research. A European Space Sciences Board has now been formed under the formal auspices of the European Science Foundation with Sir Harrie Massey as chairman.

It acts as a clearing house for research ideas in conjunction with the US Academy of Sciences' Board and looks ahead to acting as a joint negotiating body for the West for broad cooperative programmes with the Soviet Union on a range of topics better pursued on a synoptic or systematic basis than from discrete and occasional national spacecraft. The idea of an international space observatory seems particularly to recommend itself to Sagdeyev and his institute colleagues at present. The European Space Sciences Board holds its next meeting—the third—in London in the middle of September, and expects to clarify some of these possibilities. □

correspondence

The WHO and mosquitoes

SIR,—We have read with interest your editorial (July 31) on the allegations made by a section of the Indian press and the Public Accounts Committee of the Indian Parliament against the Delhi-based Research Unit on Genetic Control of Mosquitoes whose activities were jointly implemented and supervised by the World Health Organisation and the Indian Council of Medical Research (ICMR).

With regard to the technical aspects of this controversy, you have highlighted the crucial fact that the authors of the allegations failed to read the abundant literature issued by the research unit, either reports or publications, which were made freely available to them, and that they used logic so tenuous that it does not stand up to unprejudiced examination. We might add that they did not even take the trouble to discuss the controversial points with the many Indian scientists carrying out research at the unit.

We should like, however, to complement your analysis by supplying some additional information on other aspects of the controversy, for which the only documentation that you had was that presented by the authors of the allegations.

Dr Jayaraman's assertion that he was refused information on the project by the WHO because "it was sensitive to the Indian press" does not represent the truth. On the contrary, all co-operation was extended to him and a meeting was immediately arranged under the chairmanship of the Director-General of the ICMR with the Director of the National Institute of Communicable Diseases of India, the unit's Project Leader and Dr Pal of the WHO's Vector Biology and Control Unit at Headquarters. The Chairman invited Dr Jayaraman to raise any questions about the unit's research after he had a chance to see the special issue of the *Journal of Communicable Diseases* devoted to papers on the unit's work. It was most unfortunate that Dr Jayaraman never availed himself of this offer; had he done so, the fallacies in his subsequent published statements might have been avoided. You have also quoted Dr Jayaraman's allegation that he was "indirectly sounded out for a job as an Information Officer at WHO Headquarters", the implication being that this was done in an effort to appease him. No such offer was made

to him; what was offered was the full cooperation of the WHO information services in the preparation of his article. It is noteworthy that Dr Jayaraman did not make this allegation in the press, but only before the Parliamentary Committee.

With reference to the use of chemosterilised mosquitoes, your presentation of the situation is not entirely accurate. The unit's statement that thiotepa residues break down very rapidly in the bodies of mosquitos is not a mere "claim" but is based on investigations, the results of which have been published (LaBrecque, G. C., Bowman, M. C., Patterson, R. S., and Seawright, J. A., *Bull. Wld Hlth Org.*, **47**, 675-676; 1972). The unit's statement that drinking-water wells were never used for the release of chemosterilised pupae is factually correct. Apart from considerations of safety, the use of drinking-water wells (in which *Culex fatigans* does not normally breed) would have defeated the aim of releasing sterile males at the natural breeding sites, which in the area and season concerned are disused irrigation wells. The toxic effects of the well water on laboratory-reared pupae was discovered not because these were released in the wells, but because treated pupae were placed for emergence in this water in containers which in the initial stages were floating and later suspended above the water.

You sharply criticise WHO's handling of public relations during the controversy. We may point out that as an international organisation the WHO does not make any statement which could be construed as intervention in an internal dispute or a matter falling within the domestic jurisdiction of a member state. So long as this was the case, and the question was under investigation by a Parliamentary Committee in India, it was not considered appropriate to publish on this matter.

Finally, your statement that the WHO has "pulled out" may leave a false impression with your readers. The original agreement between the government of India and the WHO establishing the research unit was for a period of six years, which expired on June 30, 1975. The unit developed much essential methodology, carried out several small scale field trials and assisted in the creation of a core group of Indian scientists fully conversant with all the aspects of the research. What is left to be done is to carry out

large scale feasibility studies of new vector control methodology in areas of southern India endemic for mosquito-borne diseases, which does not require the assistance of full-time WHO staff members. It is anticipated that this work will be carried out under Indian leadership now that the WHO has handed over the unit to the Indian Council of Medical Research on the appointed date, with continued WHO technical advice and assistance if requested.

Yours faithfully,

F. J. TOMICHE

World Health Organisation, Geneva

SIR,—Your leader "Oh, New Delhi; Oh, Geneva" (July 31) might as well have been written by the World Health Organisation's Public Relations staff whom you hold responsible for the bad handling of the Indian press that ultimately, according to you, led to the closure of the Research Unit on Genetic Control of Mosquitoes (GCMU) in New Delhi.

The intention of this letter is not to highlight all the crucial omissions you had made (that would make the letter long) but only to correct a few statements which apparently have been taken out of the handout the GCMU had prepared in defence of its project.

You have dismissed the six-volume Stockholm International Peace Research Institute (SIPRI) series on chemical and biological warfare (CBW) in one sentence by saying that SIPRI "has reported that biological warfare (BW) could be conducted with infected mosquitoes."

The SIPRI series in fact says a lot more on entomological warfare. Like genetic control, it is also in the research stage. Our allegation that data gathered by the GCMU on mosquitoes can help BW research is supported by SIPRI which says that ecological data of mosquitos and dispersal data obtained from field trials are useful in BW.

It is well known that only female mosquitoes pick up and transmit viruses. Your categorical statement that GCMU's "work has been exclusively concerned with males" is however, incorrect. The GCMU was to have released at least 2,000 females a day at Sonapat. The male-female sexing error, as claimed by the GCMU, is 0.25% and in practice it may be higher.

Second, dealing predominantly with male mosquitoes need not be a great handicap. A specialist in mosquito ecology can obtain the dispersal pattern of females of a species of mosquito by extrapolating the dispersal data of males.

Your leader gives an erroneous impression that we were the only two "to level accusations of BW." Other witnesses who appeared before the Parliamentary Public Accounts Committee (PAC) belonged to the health and defence ministries. Of course they will not accuse themselves. But if you had carefully read the PAC report you would have noticed statements made by health officials admitting that both the GCMU project and the Jodhpur project (another WHO venture about which your leader is silent) could provide data useful in BW.

You may be "revolted" by the thought that the US government was using an intermediate organisation (WHO) to learn how to infect the Indians with yellow fever. Do you know that the USA was alleged to have used an FAO expert (an Indian) in 1970 to sabotage Cuban sugar cane research?

Do you know that a US expert working at the Indian Agricultural Research Institute (IARI) here was caught at the India-Pakistan border in 1971 with his luggage containing wheat germ plasm collected by the IARI?

It was unfortunate that articles criticising the GCMU and other projects were interpreted to be anti-American. This was never intended. The bird migration studies supported by the GCMU and the US army at the Bombay Natural History Society exemplified the casual attitude of some to foreign funded research. But many other countries have set up collaborative projects covering agriculture, livestock and the continental shelf.

Although we journalists appeared before the PAC late in the day, the same questions put to us were also put to official witnesses and they were given every opportunity to give answers.

You seem to regard the WHO pulling out from an "important" research project as bad. Bad for whom? Not for India which needs to tackle malaria now and not 40 years hence when genetic control may become viable scientifically—but not economically.

As far as filariasis is concerned, an expert report to the Indian Council of Medical Research had clearly said in 1971 that the strategy is not elimination of the vector *Culex fatigans*—but protection with drugs of the vulnerable population below the age

of 21 in the endemic regions.

Chikungunya and dengue are not major health problems in India (as they were made out to be during the GCMU controversy) and control of *Aedes aegypti* has the least priority. That dengue gives cross protection against yellow fever is so strongly established (Theiler, Max, *Arthropod borne diseases in vertebrates*; 1973) that grand projects to eradicate *Aedes aegypti*—even though foreign-financed and with WHO sanction—can be only anything but scientific.

Arguments that yellow fever did not strike after elimination of *Aedes aegypti* from Poona in 1953, are too weak to disprove Max Theiler's conclusion that a person immune to dengue is automatically immune to yellow fever. Such arguments are too childish to be taken seriously by the health department of any country. Yellow fever did not come to Poona perhaps because other vectors of yellow fever were also not present, or there were missing links in the epidemiological chain.

If the closing of the GCMU causes concern to British scientists they may well restart the project around London. It is better that sophisticated technologies (genetic manipulation is one) are first tried and developed in advanced countries before they are recommended to developing countries.

Your worries that India will find few opportunities to collaborate with the rest of the scientific world are unwarranted. Right now India collaborates with some 40 countries. Almost every month some science agreement or other is signed. Under such circumstances it is only natural for the PAC to recommend that foreign research in certain areas like weather modification, and oceanography should be scrutinised from the security angle.

The PAC has not banned foreign collaboration in science but has asked for the closer participation of Indian scientists who are at present given subordinate roles and not decision-making or project-management roles in foreign-funded research. The PAC has also called for thorough scrutiny by a governmental clearing body as you yourself have admitted that "almost everything has its military aspect."

We would like to ask, for instance, if the British government will allow a team of foreign scientists to go about surveying Britain's continental shelf, causing artificial earthquakes in "search of minerals", or releasing new strains of pigeons in Trafalgar Square?

C. RAGHAVAN
K. S. JAYARAMAN

Press Trust of India Ltd.
New Delhi, India

Unwelcome error

SIR,—Something went a little wrong, I am afraid, in the box headed "Wellcome Foundation" in your guide "How Britain Runs its Science" (August 28).

The Wellcome Foundation Limited is the parent company of a British based pharmaceutical group trading as Burroughs Wellcome, Coopers and Calmic around the world. Its chairman is Mr A. A. Gray. Like any other pharmaceutical group it spends money on its own research and development.

All its shares are owned by a registered charity, The Wellcome Trust, which under the Will of Sir Henry Wellcome, who died in 1936, applies all moneys it receives, as sole shareholder, to the support of medical and allied research in universities and hospitals around the world. The Director of The Wellcome Trust is Dr P. O. Williams, and I think it is the Trust rather than the foundation that should have been featured in your guide.

The figure you gave for research grants is actually the foundation's expenditure on research and development for the year ended August 31, 1974.

Yours faithfully,

BASIL SAUNDERS

The Wellcome Foundation Ltd,
London, UK

ED—Indeed the Wellcome Trust, rather than the foundation, ought to have been featured in our guide. Before anyone starts applying for a slice of the £11.5 million which the foundation allocated to research and development last year, we had better point out that the trust's spending on research assistance (1972–74) was £1,133,463.



A hundred years ago

THE German Scientific and Medical Association was opened at Graz on the 17th inst. Lieut. Weyprecht, of the recent Austrian Arctic Expedition, made a speech deprecating all past Arctic expeditions as adventurous and valueless because they constituted an international rivalry that resulted only in giving names to some ice-bound islands. The speaker, amid general applause, expounded a new programme for making Arctic expeditions more fruitful for natural science, and to enable poorer countries to undertake such expeditions from *Nature*, 12, 260; September, 23, 1875

news and views

It has been little more than a year since the first publication (Olins and Olins, *Science*, **183**, 330; 1974) of electron micrographs showing the "string of beads" structure of chromatin. During this period, much other evidence has been presented (*Nature*, **254**, 651; 1975) that, at this lowest level of organisation, the DNA of chromatin is folded together with histones to form morphologically repeating units (called "bodies" or nucleosomes) involving about 190 base pairs of DNA and eight histone molecules each. We still do not know the nature of this folding—the conformation of DNA within the particle, the structure of the histone complexes which constrain the DNA, and whether or not the histone content and DNA conformation of all particles are identical. These questions are examined in several new papers.

DNA folding

Valuable information about DNA folding within the nucleosome has been obtained by chemical methods. A recent paper by Germond *et al.* (*Proc. natn Acad Sci. U.S.A.*, **72**, 1843; 1975) examines the properties of complexes obtained by associating histones with covalently closed circular DNA isolated from simian virus 40 (SV40). Earlier papers (Olins, *J Cell Biol.*, **64**, 528; 1975; Oudet *et al.* *Cell*, **4**, 281; 1975) had shown that the beaded-string structure could be generated by reconstituting DNA with a mixture of each of the four histones H2A, H2B, H3 and H4. By making use of SV40 DNA in the reconstitution experiments, Germond *et al.* are now able to study the effects of nucleosomes on DNA superhelix formation. The experiments depend on the use of an "untwisting extract" isolated from Krebs II ascites cells, which contains an enzymic activity capable of removing the superhelical content of circular covalently closed DNA, leaving a relaxed product that is still a closed circle. Germond *et al.* formed a series of complexes between relaxed circular SV40 DNA and increasing quantities of a mixture of the four histones and treated the complexes with untwisting extract. When DNA was then freed of histone and its superhelical content determined, it was found that superhelix had been generated. The number of negative superhelical turns present in the DNA at the end of the experiment was equal to the number of nucleosomes per molecule observed for the corresponding

String of pearls

from Gary Felsenfeld

nucleoprotein complex in the electron microscope.

These results show that the formation of each nucleosome involves creation of one superhelical turn or its topological equivalent, but do not tell us what the actual DNA conformation is within the nucleosome, since the effect of one negative superhelical turn can also be generated by unwinding the DNA double helix one full turn, or by a series of negative and positive superhelical turns which partially cancel each other. Structural considerations place some limits on speculation, however. A single regular superhelical turn of 120 Å diameter and 110–120 Å pitch will not accommodate the amount of DNA found in the nucleosome but other superhelices can be constructed (Baldwin *et al.*, *Nature*, **253**, 245; 1975). Crick and Klug (*Nature*, **255**, 530; 1975) have proposed a class of models for the nucleosome in which the DNA, rather than being smoothly bent, is kinked at intervals in order to wrap around a core of histones. One specific model that they propose involves a 95–100° kink every 20 base pairs, generating between two and three left-handed superhelical turns in 190 base pairs. This model appears to be outside the range suggested by the results of Germond *et al.*, but it is easy to imagine variations of the model which would fit better, since the sense and pitch of the kinked supercoil are sensitive to the number of base pairs between kinks.

There is some question also about what fraction of the 190 base pair repeating DNA subunit is actually in the "bead", and what fraction is in the "string". Several investigators (Van Holde *et al.*, *Biochem. biophys. Res. Commun.*, **60**, 1365; 1974; Solner-Webb and Felsenfeld, *Biochemistry*, **14**, 2915; 1975; Axel, *Biochemistry*, **14**, 2921; 1975) interpret the electron microscopic and nuclease digestion results as indicating the presence of a string occupying perhaps 50 base pairs of the subunit. This part of the DNA, seen in the microscope as a fibre 15–20 Å in diameter, is probably not supercoiled, though it could be thinly covered by a

histone chain, perhaps wound in one of the grooves of the double helix. Noll *et al.* (*Science*, **187**, 1203; 1975) however, take the position that all of the DNA in native chromatin is wound into the nucleosome, and that the strings between nucleosomes are seen only if chromatin is subject to disruptive shearing forces. The electron microscopic studies of Griffith (*Science*, **187**, 1202; 1975) on naturally occurring SV40 DNA–histone complexes indicate that the string regions are visible at low ionic strength, but disappear from view as salt concentration is raised to 0.15 M, suggesting that these regions are in some way a unique part of the unit structure.

Arrangement of histones

The other half of the nucleosome structure problem has to do with the arrangement of the histones. The known histone content of chromatin is approximately consistent (Kornberg, *Science*, **184**, 868; 1975) with a repeating unit containing eight histones (two each of H2A, H2B, H3 and H4) for each DNA repeat of 190 base pairs. An (H3H4)₂ tetramer is present in histone fractions prepared by salt dissociation of chromatin (Kornberg, *loc. cit.* Kornberg and Thomas, *Science*, **184**, 865; 1974). Kornberg proposed that the chromatin structure was built upon a core of this tetramer, to which were added two H2A–H2B dimers to complete the octameric structure. Since histones H3 and H4 spontaneously form dimers and tetramers in solution (Roark *et al.*, *Biochem. biophys. Res. Commun.*, **59**, 542; 1974; D'Anna and Isenberg, *Biochemistry*, **13**, 4992; 1974) the presence of such complexes after release from DNA does not constitute conclusive proof of their arrangement within the chromatin structure. A more satisfactory approach to the problem is to treat chromatin with protein crosslinking reagents, and identify the histone oligomers which result, a method that has proved valuable in the study of other nucleoprotein complexes. Martinson and McCarthy (*Biochemistry*, **14**, 1073; 1975) have used tetranitromethane to demonstrate the presence of an H2B–H4 dimer in chromatin; they find that this dimer also forms when histones are reconstituted with DNA, but only when H2A is also present. VanLente *et al.* (*Cell*, **5**, 45; 1975) have treated nuclei and chromatin with formaldehyde, producing dimers of H2B with H4, and H2A with

H2B, while Bonner and Pollard (*Biochem. biophys. Res. Commun.*, **64**, 282; 1975) have generated an H3-H4 dimer using carbodiimide. Thomas and Kornberg (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2626; 1975) are the first to report the isolation of cross-linked histone octamers. Chromatin is treated with dimethyl suberimidate (Me_2Sub) or dithiobis (succinimidyl propionate) (Lomant's reagent). At pH 9, reaction with Me_2Sub produces a series of protein oligomers ranging in molecular weight from dimer to octamer, but there is little or no oligomer of larger size. Using Lomant's reagent, the principal low molecular weight product is octamer, but higher molecular weight products (16-mer and higher) also occur. It is not yet determined whether the octamer represents a single species or a mixture of several complexes varying in histone content, but of about the same molecular weight. Examination of the dimers produced as reaction intermediates by Me_2Sub has led to identification of $(\text{H3})_2$, H3-H4, H2B-H4, H2A-H4, and probably H2A-H2B. The same pairs were obtained when chromatin monomers (isolated single nucleosomes) were crosslinked, suggesting that these pairs are representative of short-range interactions within the monomer.

Are all nucleosomes identical in histone content and arrangement? That

is the simplest and most reasonable working hypothesis, but it remains to be demonstrated. If there is some variation in the histone content of nucleosomes, it must be subject to certain rules of substitution, since nucleosomes seem to be fairly homogeneous in protein mass, and there is some variation among histones in molecular weight. If the nucleosomes are all identical in histone content, it is still possible that variations in internal folding or arrangement exist. This is an unattractive hypothesis because multimeric protein complexes tend to have unique arrangements. On the other hand, naturally occurring histone modifications such as phosphorylation are known to have a major effect on physical properties that might affect the way histones fold or interact.

An attempt to probe the internal arrangement of histones has been made by Weintraub (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1212; 1975) who has examined the products obtained when chromatin is digested extensively with staphylococcal nuclease, and then with trypsin. The trypsin digestion removes only 20 to 30 amino acid residues from the histone N-terminals; when the resulting nucleoprotein fragments are examined by electrophoresis on acrylamide gels, eight discrete nucleoprotein bands are observed. Four of these can be shown to carry C-terminal

portions of histones H2A, H2B, H3 and H4, one carries only H3 and H4 C-terminals, and three bands are altogether free of protein, presumably because they carried the trypsin-sensitive N-terminals. If chromatin is digested first with trypsin and then with nuclease, results consistent with this hypothesis are obtained: the protein-free bands generated by nuclease-trypsin treatment are not present in the trypsin-nuclease digest because the N-terminal attachments which protect these bands against nuclease attack have been destroyed. Weintraub's results reflect a well-defined architecture of histone-DNA attachment sites within the nucleosome. But the number and size of the fragments is too large to have arisen from a single kind of nucleosome particle undergoing degradation along a single pathway. It is possible that there are several paths of degradation leading from the same starting point to different sets of products, so that the appearance of multiple fragments in the digest cannot be taken as unequivocal evidence for heterogeneity of nucleosomes. Proof that there is only one kind of nucleosome, however, also awaits definitive quantitative experiments. If nucleosomes are homogeneous, the hope exists that they can be crystallised, and their structure determined by the direct methods of X-ray diffraction. □

YET another satellite of Jupiter has been discovered, the thirteenth. Unlike new comets and asteroids, which seem to pop up at frequent intervals, new satellites are much less common, Jupiter XII being found in 1951 and Saturn X in 1966. Needless to say planetary discoveries are rarer still, coming at a rate of about one every 75 years (Uranus 1781, Neptune 1846 and Pluto 1930).

The discovery, observations and attempts to determine the orbit of Jupiter XIII are discussed in a recent paper by four American astronomers Kowal, Aksnes, Marsden and Roemer (*Astr. J.*, **80**, 460; 1975). They used the 122-cm Schmidt telescope at Palomar Mountain and on three consecutive nights during September 1974 photographed a $6^\circ \times 6^\circ$ field centred on Jupiter. The plates were exposed for 120 min, the telescope being guided to follow Jupiter. At the time Jupiter was near opposition (at its closest to Earth, about 3.98 AU away) and of magnitude -2.5 . The authors estimated that the faintest images that could be detected on each plate, for an object sharing Jupiter's motion, were of magnitude about $+0.22$, equivalent to a brightness 6×10^9 times less than Jupiter's.

The satellite was discovered during

the course of 'blinking' the plates—a process in which two plates are looked at in quick succession and any image which has moved relative to the fixed background stars appears to jump to and fro as each plate is successively observed. Accurate measurements of the first three plates showed that the object's motion during September 11–13 could lie on two possible orbits, a direct joviocentric one and a heliocentric orbit similar to that of an asteroid. This confusion was only resolved after seven more plates were taken, the latest on December 12 when Jupiter was more than three

Jupiter XIII

from David W. Hughes

months past opposition and at a distance of 5.03 AU. Four of these were obtained using an image tube attached to the Steward Observatory's 229-cm reflector on Kitt Peak, the plates requiring only 1.5 min exposures.

Just considering their distances from Jupiter, the Jovian satellites seem to fall into three distinct groups, (I-V), (VI, VII and X) and VIII, IX, XI and XII, these being within 1.8, around 12 and between 21 and 24 million km from the planet with wide

empty gaps in between. The new satellite XIII is a member of the second group having an orbit with semi-major axis 0.074 AU (11.1 million km), eccentricity 0.147, inclination 26.7° and period 239 d. Its photographic mean magnitude is close to 21, about 1.5 magnitudes fainter than Jupiter XII. The radius of Jupiter XII is estimated to be around 8 km so Jupiter XIII is probably smaller. Whether these outer satellites are true-born children of Jupiter, formed in the same epoch, or whether they have been adopted at some later date remains as yet uncertain. Jupiter's seven outer satellites all have radii less than 12 km and could easily be captured asteroids probably with shapes and surface features like Phobos and Deimos, the moons of Mars.

Interestingly, Kowal *et al.* state that for the next few years there is about an even chance that each annual Jovian opposition could see the discovery of a new satellite, of comparable brightness to Jupiter XIII. The satellite would however have to be near its extreme elongation east or west of Jupiter. The opposition in 1975 (around October 13) is especially favourable as Jupiter is at the perihelion of its orbit and only 3.96 AU from the Sun.

From nuclear β decay to the charges of quarks

from R. J. Blin-Stoyle

ELEMENTARY particle or high energy physics became established as a subject in its own right during the immediate postwar years and its varied and often surprising revelations about the fundamental structure of matter have continued unabated since then. It developed from research into the properties of atomic nuclei; but whereas most nuclear properties can be unravelled using low energy (tens of MeV) accelerators and many body theory, the creation of new particles and the study of their properties requires vastly higher energies (tens of thousands of MeV and more) and has led to the development of most elegant but continually evolving theories. Nevertheless, low energy nuclear physics continues to make important contributions to our understanding of the physics of elementary particles. This brief commentary is intended to illustrate the foregoing by taking as an example some recent deductions of Wilkinson (published in this issue of *Nature*, page 189) based on very careful measurements of nuclear β -decay rates and energies.

Quark-parton model

To set the scene it must first be stated that research in high energy physics has led to the discovery of many hundreds of elementary particles and to the elucidation of their interactions and properties. In particular it has become clear that their behaviour is conditioned by three basic interactions which, in descending order of strength are known as strong, electromagnetic and weak. The strong interaction is responsible, for example, for the powerful forces that hold the nucleus together; the electromagnetic interaction is well known and manifests itself in all electromagnetic phenomena; the weak interaction is responsible, among other things, for the radioactive decay of elementary particles and, in particular, for the process of nuclear β decay. But the main properties of particles are naturally determined by the dominating strong interaction and at the present time it seems likely that the nature of the strong interaction is such that particles which experience it, and this includes the vast majority, are all, in some sense, composite structures of a very small set of basic particles (or their anti-particles) called quarks. For example, the component particles of the nucleus, namely neutrons and protons, are regarded as consisting of three quarks which, incidentally, may further be identified with the point-like objects known as partons indicated by some

experiments to be constituents of elementary particles.

What Wilkinson has done is to assume the correctness of the quark-parton model and then to derive the charge of these constituent particles from β -decay data. This is a remarkable connection to make and inevitably intermediary assumptions and hypotheses have to be made in achieving it. But what is important is not so much the result and the extent to which it can be considered reliable, but the fact that the connection can be made and can clearly be improved and developed in the future.

Briefly the steps taken are as follows. They start naturally from basic input experimental data—the lifetimes and energy release of certain particularly simple nuclear β decays known as superallowed transitions. The measurements involved which, it must be stressed, have been carried out to extremely high accuracy, have been the preoccupation of Joan Freeman and her colleagues at Harwell over the last decade or so and more recently by other researchers in Canada and the USA. From these data it is then possible to deduce the strength of the β -decay interaction. Fortunately the deduction is very largely independent of the nuclear structure details for the decays concerned—indeed, this is why they were chosen for study. The strength is measured by a coupling constant $g_{\beta V}$ (where V signifies that we are dealing with the vector part of the β -decay interaction), which has a similar role to the proton charge e characterising the strength of the electromagnetic interaction. In fact, the similarity is more than trivial and in deducing the value of $g_{\beta V}$ it is assumed that the weak ‘current’ responsible for the superallowed β decays is conserved (the well established conserved vector current hypothesis) in analogy to the electromagnetic current. The only difference is that it is a charged current and causes the decaying nucleus to change its charge when it decays (since it emits an electron or positron) whereas the electromagnetic current is neutral so that a nucleus decaying electromagnetically retains its charge (since it emits a photon).

Cabibbo universality

Now it is a further hypothesis of weak interaction physics that it is universal in the sense that all weak interaction processes are governed by essentially the same coupling constant g to which $g_{\beta V}$ must therefore be related. The hypothesised relation is simple, namely, $g_{\beta V} = g \cos \theta_c$ where

θ_c is known as the Cabibbo angle. Here it should be remarked that nuclear β decay conserves ‘hypercharge’, a quantity rather analogous to ordinary electric charge, but that in those decays of elementary particles which do not conserve it the relevant coupling constant is $g \sin \theta_c$. Thus, this ‘Cabibbo universality’ simply involves the sharing of the strength g between these two different categories of β decay. The value of θ_c can be determined with quite reasonable accuracy from data on hypercharge non-conserving β decays and so, using the expression $g_{\beta V} = g \cos \theta_c$ and knowing $g_{\beta V}$ and θ_c , the value of g can be obtained.

However, assuming the universality of the weak interaction already referred to, g is also the coupling constant governing the decay rate of the μ -meson into an electron, neutrino and antineutrino. This decay is unique and simple to treat theoretically since, in contradistinction to the β -decay situation, none of the particles involved experience strong interactions. In addition its rate has been measured accurately so that a value for g can be obtained very straightforwardly. However, before comparing the values of g from β - and μ -decay data, corrections due to electromagnetic effects have to be made to both values. These are known as electromagnetic radiative corrections and result from the modification of the decay rates due to the electromagnetic interactions between the particles involved in the decays. The difference in these corrections for the two decays, and this is all that concerns us here, is related to two quantities. First, being an electromagnetic effect, the β -decay radiative correction naturally depends on the charges of the constituent quarks of the neutrons and protons involved in the decay. Second, for both β - and μ -decay the correction depends on a characteristic mass M , and here we come to the final hypothesis to be introduced.

Over the last few years theories have been developed which unify the weak and electromagnetic interactions. One particularly successful version is that of Salam and Weinberg and is well supported by recent neutrino scattering experiments which establish the presence of neutral weak currents (as distinct from the charged currents already mentioned). Just as the neutral electromagnetic current couples to the photon, so the neutral weak current couples to a very heavy uncharged (that is, neutral) meson whose mass can be determined from the neutrino

scattering experiments. Assuming the validity of the Salam-Weinberg theory it then transpires that the mass M arising in the β - and μ -decay electromagnetic radiative corrections is just that of the neutral meson.

The connection is now complete and all that remains is to choose the quark charges so that the values of g derived independently from the β - and μ -decay data agree with one another. Suffice it to say that Wilkinson's results are consistent with one third integral charges for quarks as originally suggested by Gell-Mann and Ne'eman and not with integral charges as suggested recently.

As stressed earlier in this commentary the result, although extremely interesting in itself, does rest on a rather complex edifice of hypotheses

and theories some components of which are perhaps a little delicate. What is important is that it has been shown how quite fundamental information about properties of elementary particles can be derived from very accurate measurements within the framework of conventional low energy physics. There are other properties of elementary particles and their interactions that are being studied in similar ways (such as time reversal invariance, parity violating nuclear forces, exotic interactions) and there are those of us who feel that, quite apart from achieving a full understanding of the nucleus as a many-body system, there are these other important reasons for continuing detailed studies of nuclear phenomena. \square

F_c receptors and Ia antigens: a postscript

from Robert S. Kerbel

Is there a unique relationship between Ia antigens and F_c receptors on the surface of B lymphocytes? As discussed in a recent *News and Views* article (*Nature*, **255**, 576; 1975), an affirmative answer to this question would almost certainly have far-reaching implications for various aspects of the immune response and its regulation: these have been succinctly summarised (*Transplant Rev.*, **23**, 159; 1975) by the same investigators who in fact first presented evidence for an identity or close association between F_c receptors and Ia antigens on B cells (Dickler and Sachs, *J. exp. Med.*, **140**, 779; 1974).

To summarise briefly: Ia antigens are a system of alloantigens controlled by the I (immune response) region of the major histocompatibility gene complex (H-2) in the mouse. They are expressed in quantitatively significant amounts on B cells, and appear to be responsible for, among other things, the generation of mixed lymphocyte responses in culture and the generation of *in vivo* graft-versus-host responses. The F_c receptor is a cell-surface component which interacts with the F_c portion of certain immunoglobulins and is found on a wide variety of cells, including B lymphocytes. It has potential significance in a spectrum of immunological phenomena. Dickler and Sachs suggested that a special relationship may exist between Ia antigens and F_c receptors on the basis of experiments in which the binding of aggregated immunoglobulin (Ig) to B cell F_c receptors was inhibited by anti-Ia antibodies but not by anti-H2K, anti-H2D, or anti-Ig antibodies.

Schirmmacher *et al.* (*J. exp. Med.*, **141**, 1201; 1975) however, found no evidence for a unique association, on

the basis of results obtained from two alternative assays both of which utilised the same basic idea of assessing the relationship of cell surface components to F_c receptors by the ability of antibody (against these components) to inhibit F_c receptor binding. As was pointed out, these results, although they provided no support for a unique association theory, did not formally disprove the notion either. More direct evidence is needed to settle this controversy: in this issue of *Nature*, Rask, Klareskog, Ostberg and Peterson (page 231) have tried to provide such evidence by isolating B cell F_c receptors and examining some of their properties. Their results appear to show that neither an identity nor close association exists between F_c receptors and Ia antigens.

Rask *et al.* prepared soluble macromolecules from crude membrane fractions of mouse spleen cells which were then subjected to affinity chromatography on a column of sepharose beads to which heat-aggregated IgG molecules had been covalently attached. Any soluble F_c receptors would be expected to bind to the aggregated Ig which could then be eluted. When such eluted material was subjected to polyacrylamide gel electrophoresis, Rask *et al.* found three types of sialic-acid containing polypeptide chains with apparent molecular weights of about 65,000, 18,000, and 15,000. Evidence was cited by the authors which indicated the three components behaved like F_c receptors in that they had affinity for soluble immune complexes.

The remainder of the experiments involved the use of rabbit antisera raised separately against the 65,000 dalton material and the mixture of the 18,000 and 15,000 dalton components.

When these antisera were incubated with mouse lymphoid cells they appeared to react only with B cells which would be expected if the antibodies had specificity for F_c receptors. IgG and $F_{(ab')_2}$ fractions of these antisera also possessed the ability to inhibit the uptake of aggregated human IgG on to B cells.

The authors then examined the possibility of a close relationship existing between Ia antigens and F_c receptors by use of the so-called 'lysostripping' method, which is based on the assumption that independent molecules float freely in the cell membrane, and can be redistributed by antibodies into 'caps' on the cell from an originally diffuse or patchy distribution. The cells then become specifically resistant to complement-dependent lysis when further incubated with antibodies directed against the capped antigens. Antibodies against other cell surface components however retain their full cytotoxic ability provided an intimate relationship does not exist between them and the capped antigens. If such a relationship does exist, these other surface components can be 'co-capped' as well, resulting in resistance to complement-dependent lysis. The principle of co-capping of cell-surface components was one of the first methods used, for example, to show that a close physical relationship exists between histocompatibility antigens and β -2 microglobulin (Poulik *et al.*, *Science*, **182**, 1352; 1973).

When Rask *et al.* incubated spleen B cells with their presumptive anti- F_c receptor antisera so as to induce capping, the cells subsequently became resistant to lysis mediated by the same antisera; but when tested with anti-Ia sera, the cells were apparently killed, that is the Ia antigens did not appear to co-cap with the F_c receptors. The authors conclude that no unique association exists between Ia antigens and F_c receptors although they may lie adjacent to one another in the cell membrane.

An attempt to help resolve another controversy surrounding Ia antigens; their presence or absence on T cells, also appears in this issue of *Nature* (page 230) by Goding, White and Marchalonis. Previous reports have indicated the presence of Ia antigens only on B cells, or on both B and T cells. The reasons for the discrepancies are unclear but may be partially due to the nature and potency of the anti-Ia sera used in various studies. In any case, Goding *et al.* demonstrate the specific immunological precipitation of small amounts of Ia antigens from extracts of surface radioiodinated thymocytes using an anti-Ia serum of very high titre. The results are consistent with recent serological data showing the

presence of very small amounts of Ia antigens on less than 50% of thymocytes, a finding made possible by the use of a fluorescence-activated electronic cell sorter (Fathman *et al.*, *J. Immun.*, **115**, 584; 1975). More conventional procedures such as immunofluorescence, cytotoxicity, and absorption of antisera, do not allow detection of Ia antigens on thymocytes although they readily do so on B cells.

The presence of small amounts of Ia antigens on thymocytes is more than vaguely similar to the story of Ig on thymocytes and T cells. Since it seems that the presence of Ig on T cells is sometimes a result of its passive adsorption on to the surfaces of the cells, and not to its endogenous synthesis, an obvious point arises as to whether the same is true for Ia antigens and thymocytes. Indeed, it has been demonstrated by Vitetta *et al.* (*J. Immunogenet.*, **1**, 82; 1974) that, in contrast to H2 antigens, soluble Ia antigens are readily shed into incubation medium containing Ia-positive lymphocytes. In addition, passage of Ia-positive lymphocytes down a column of nylon wool (a procedure used to deplete cell populations of B cells) seems to remove selectively some of the Ia antigens from the surface of the B cells (Schultz *et al.*, *Cell. Immun.*, **16**, 125; 1975, and Fathman *et al.*, *J. Immun.*, **115**, 584; 1975).

These intriguing findings, besides being of some significance themselves with respect to Ia antigen function, will no doubt ensure perpetuation of the controversy surrounding the presence and relationship of Ia antigens to T cells. □

More new particles

In the article 'More new particles' which appeared in News and Views last week an editorial change implied that a section was a full account of work discussed at the recent Lepton-Photon conference at Stanford; the article was in fact received before the Stanford conference.

Nuclear states of high spin

from P. E. Hodgson

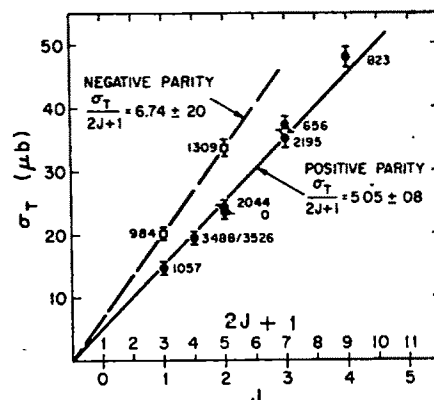
ONE of the most powerful ways of determining the spins of nuclear states is by one-nucleon transfer reactions: the angular distribution of the emitted particles is characteristic of the angular momentum transfer, and this usually suffices, sometimes in association with other data, to fix the spin of the final state in the residual nucleus.

This method works best for low

spins, corresponding to angular momentum transfers of 0, 1 and 2. For higher spins the angular distribution is not so characteristic, and the reaction may be forbidden by the spin selection rules.

Among the methods applicable to high spin states the ($^7\text{Li}, p$) reaction is proving useful, and a recent paper by Bishop and Fortune (*Phys. Rev. Lett.*, **34**, 1350; 1975) provides a good illustration of this.

At low energies on light nuclei the ($^7\text{Li}, p$) reaction proceeds predominantly through the compound nucleus. The ^7Li is captured by the target nucleus to form a compound system which then can decay by proton emission. It is then found that the total cross section for the reaction is closely proportional to $(2J+1)$, where J is the spin of the final nuclear state. This relation has been tested for a large number of states, and can be used with some confidence to determine unknown spins.



Total cross sections for the $^{14}\text{N}(^7\text{Li}, p)^{20}\text{F}$ reaction to a number of positive and negative parity states of ^{20}F plotted as a function of $(2J+1)$.

Bishop and Fortune studied the reaction $^{14}\text{N}(^7\text{Li}, p)^{20}\text{F}$, and their results for some positive and negative parity states of ^{20}F with known spins are shown in the figure. The cross sections are closely proportional to $(2J+1)$ and the ratio of the total cross section to $(2J+1)$ is 5.05 ± 0.08 for the positive parity states and 6.74 ± 0.20 for the negative parity states. The proportionality is so closely followed that it is possible to use the total cross sections for the ($^7\text{Li}, p$) reaction to determine the spins of some other states. The identification can be strengthened by seeing how well the state fits into the rotational band structure of the low-lying states of ^{20}F .

The method used is simply to calculate the spin from the expression $\frac{1}{2}[(\sigma_T/5.05)-1]$ for positive parity states and $\frac{1}{2}[(\sigma_T/6.74)-1]$ for negative parity

states and to compare these spin values with those of the possible states that fit into the band structure.

For the state at 2.87 MeV for example, the calculated spins are 3.5 for a positive parity state and 2.5 for negative parity. For the known band structure the assignment 3^- is considered the most likely. In a similar way one of two states at 2.97 MeV is probably identified as 4^- . Probable spin assignments are made to several other states as well.

This work shows the usefulness of the ($^7\text{Li}, p$) reaction in determining the spins of nuclear states, when used in conjunction with other techniques. □

Bird song dialects

from John R. Krebs

SONG birds, like humans, have local dialects. Regional variations in the form of territorial song have been found in such birds as chaffinches, tree-creeper, cardinals, white-crowned sparrows and song sparrows. Often dialect regions are quite small, in the order of a few miles across, and the transition between them may be very sudden. Within a dialect region all males have similar songs whereas between regions the song varies considerably. Playback experiments in which recorded songs are broadcast inside a territory have shown that males can tell the difference between songs of their own dialect and those from other regions (Lemon, *Anim. Behav.*, **15**, 538-595; 1967; Milligan and Verner, *Condor*, **73**, 208-213; 1971); territorial males react more vigorously to songs of their own dialect region.

Dialects probably originate as a result of young birds learning the songs of nearby adults soon after leaving the nest. In the white-crowned sparrow, for example, young males learn the territorial song within the first three months after leaving the nest, so that they are likely to learn the characteristic song of their birthplace.

Several workers have suggested that dialects play a role in the genetic adaptation of populations to local environmental conditions, by acting as a reproductive barrier between populations. This is based on the assumption, as yet untested, that females as well as males can recognise songs of their own dialect region, and will mate preferentially with local birds. Nottebohm (*Condor*, **71**, 299-315; 1969) found that dialect boundaries in a South American sparrow, the chingolo, correspond with changes in habitat structure, for example from grassland to forest, which is what one would expect if dialect regions represented locally

adapted populations. The most obvious prediction of this hypothesis is that dialect boundaries should coincide with genetic boundaries between populations, and this has recently been tested by Baker (*Evolution*, **29**, 226-241; 1975). Baker, working with white-crowned sparrows, used electrophoretic techniques to examine the differences between local populations in the frequency of marker genes, in the Colorado Rockies and at Point Reyes, California. The study area in California was apparently uniform open scrubland, but nevertheless there were sharp dialect boundaries associated with minor habitat features such as an old fence or a ditch. Baker examined the changes in allele frequency across one dialect boundary, and the overall picture for six loci was of quite marked changes at the boundary, with little change within either dialect region. This result seems to support the reproductive isolation hypothesis, but if the populations are adapted to local conditions, the adaptations must be rather subtle since the habitat did not obviously change at the boundary.

In the East River valley system in Colorado, Baker found that all the birds sang the same dialect, but there were changes in allele frequency at three loci associated with changes in altitude. So genetic subdivision of populations can occur without division into dialect regions. Taken together, Baker's results do not provide convincing support for the role of dialects in reproductive separation of populations, and he suggests that whether or not dialect and genetic boundaries coincide may depend on how an area is colonised. The Californian scrub habitat is occasionally devastated by fire, so that the white-crowned sparrow population is reduced to a few birds living in isolated patches of surviving scrub. Dialect regions probably arise through the subsequent spreading of these isolated groups as the habitat recovers, slight differences in song between the founder members of each group being perpetuated by cultural transmission, and slight genetic differences arising by chance. The Colorado valleys are stable and the population of sparrows does not get separated into isolated groups, hence dialect regions have not been established. Presumably genetic changes have arisen through selection in the face of gene flow.

The next step in following up Baker's study will be to find cases (for example small islands) where the colonisation and establishment of dialect differences can be tracked in detail. It remains to be seen whether or not a clearer link between genetic and dialect subdivisions will be found in species like the chingolo where dialects are associated with habitats. □

Genetic arrangement in eukaryotic DNA

from T. H. Rabbitts

THE 'amount' of DNA in eukaryotic genomes is known to be extremely large. The mammalian genome, for example, consists of around 5×10^9 base pairs (per haploid set) which would be sufficient to code for about two million proteins each containing 650 amino acids. In addition it is known that eukaryotic DNA consists of both single copy sequences and repetitive sequences (sequences present as multiple copies in the genome). Two questions currently receiving critical appraisal are how the different types of DNA sequences are arranged in the genome and how much of the genome actually carries protein coding information.

Much evidence has been accumulating in support of the general concept that in many eukaryotes a large percentage of the middle repetitive DNA (DNA sequences present in a few hundred copies per haploid genome) is interspersed amongst single-copy sequences. These single copy sequences are typically about 1,500 nucleotides long with the repetitive elements being around 300 nucleotides long. In two recent papers, Britten, Davidson and their co-workers have extended this evidence and produced a general review of DNA sequence organisation of eukaryotes. In the study by Goldberg *et al.* (*Chromosoma*, **51**, 225; 1975) the sequence arrangement of five marine invertebrates was determined by the examination of the reassociation kinetics of two different length DNA fragments (300 and 3,000 bases). Hybrid formation was assayed by hydroxyapatite fractionation so that DNA fragments carrying a repetitive sequence plus a non-repetitive sequence would kinetically appear in the repetitive part of the DNA reassociation curve. Using this method, all the genomes studied were found to possess a large portion of their single copy sequences interspersed with repeating sequences. In addition it was shown that these repetitive sequences are about 300 bases long.

Davidson *et al.* (*Chromosoma*, **51**, 253, 1975) have correlated the data on sequence organisation in a wide variety of metazoa. They conclude that the DNA of organisms from most branches of the evolutionary tree shares the property of containing both unique and repetitive sequences in at least 70% of DNA fragments 2,000 to 3,000 bases long. The only exception (for which data are available at present) seems to

be *Drosophila* DNA. A study of this DNA has been made by Manning *et al.* (*Cell* **4**, 141; 1975). These workers examined the interspersion pattern of single copy and repeating sequences using both the hydroxyapatite procedures and electron microscopy of re-associated fragments of differing length. It was observed that *Drosophila* DNA has an average distance of around 13,000 nucleotides between middle repetitive sequences. This number of bases is on average about six times as great as that found in the organisms reviewed by Davidson *et al.* In addition to the data of Manning *et al.*, randomly chosen fragments of *Drosophila* DNA have been cloned in *E. coli* and the re-association properties studied (Wensink *et al.*, *Cell*, **3**, 315; 1974; Glover *et al.*, *Cell*, **5**, 149; 1975). So far, the cloned DNA chosen for study has revealed only segments of DNA lacking internal repetition over as many as 15,000 to 27,000 bases. These data seem to prove that *Drosophila* DNA does not possess short repeating sequences interspersed with single-copy DNA. Further examples of the '*Drosophila*' type organisation may well soon be discovered.

Whether the interspersing nature of repetitive sequences in organisms such as *Xenopus* has any direct relevance to gene control is far from clear. Indeed, there are indications that not all repeating DNAs possess regulatory function since cytoplasmic messenger RNA-like molecules have been shown to hybridise to repetitive sequences in a variety of organisms. This leads to the question of how much of the genome actually carries protein coding information. Hybridisation kinetic analysis has recently been used to examine this problem quantitatively. Bishop *et al.* (*Nature*, **250**, 199-204; 1974) prepared complementary DNA (cDNA) against poly(A)-containing RNA of HeLa cell cytoplasm using reverse transcriptase. The kinetic complexity of the RNA population was then determined by hybridisation of excess RNA with cDNA. Using this method, Bishop *et al.* concluded that HeLa cell cytoplasm contains as many as 35,000 different mRNA species. This number of gene products is much higher than the number obtained from a similar study of *Drosophila* referred to by Izquierdo and Bishop and described in detail by Levy and McCarthy (*Biochemistry*, **14**, 2440; 1975). In the latter cases, an mRNA complexity around 4,000 to 7,000 species was found. These numbers compare nicely with the number of bands seen by Bridges in polytene chromosomes (*J. Hered.*, **29**, 11; 1938). What then is the function of the excess DNA which makes up the genome?

Excluding the genes for the rRNAs and tRNAs it begins to look as if a much greater complexity of nuclear

RNA exists than is found for cytoplasmic RNA. Hough *et al.* (*Cell*, **5**, 291; 1975) have studied the hybridisation of purified radioactive unique sea urchin DNA in RNA-excess reactions with gastrula nuclear RNA. These experiments indicate that around 28% of the total complexity of the sea urchin genome is represented in this nuclear RNA. This complexity of nuclear RNA is at least an order of magnitude higher than that found for sea urchin polysomal mRNA. Earlier experiments (Getz *et al.*, *Cell*, **4**, 121; 1975) with mouse Friend cells arrived at similar general conclusions. In this work, the complexity of poly(A)-containing nuclear RNA was assessed by kinetic hybridisations with cDNA. The authors conclude that at least five times more unique DNA is represented in the nuclear RNA than in the polysomal mRNA of these cells. Although the numbers involved in the papers of Hough *et al.* and Getz *et al.* are somewhat different (this difference probably being a result of the use of total nuclear RNA in the former case and poly(A)-containing nuclear RNA in the latter case), the general conclusion is the same—a larger amount of the genomic information is synthesised into nuclear RNA than is present in the cytoplasmic poly(A)-containing RNA. This disparity between the two RNA populations allows speculation regarding the function of the RNA which seems to remain in the nucleus.

Even with these detailed numerical analyses of eukaryotic gene complexity, very little is known of the fine structure of individual genes and genetic units. Studies of these structures should clarify the nature of the controlling elements and specific informational organisation in eukaryotic DNA. □

Excited atoms

from a Correspondent

The ninth international conference on "The Physics of Electronic and Atomic Collisions" was held in Seattle on July 24–30.

ATOMIC physics is finding applications in horticulture: Sir Harrie Massey, delivering the opening scientific talk of the conference, reminded delegates that the study of negative ions, in what was non-applied research in atomic physics, resulted in the method for determining levels of DDT in crops. Though that particular spin-off was successful the nuclear fusion story is a less cheerful one: the level of aid so far given to the thermonuclear fusion programme is worrying. Delegates at

the conference were aware of the immediate need for atomic scattering cross-section data to assist in the construction of a viable fusion machine. It was stressed that measured cross sections for electron scattering of highly excited and ionised atoms and molecules was needed immediately to enable a composite picture of the competing processes in the 'fusion plasma' to be made. An estimated 40% of the power losses from the injected ion beams occurs through the presence of impurity ions such as C^+ , Fe^+ , Mo^+ by way of electron capture, dielectronic recombination and bremsstrahlung losses. But the problem, as expressed by some delegates, is that "the cross section we want today isn't the one we need tomorrow".

Experimental investigations into highly excited states of atoms, for example H, Na, Xe, has advanced very rapidly since the last meeting. In addition to using charge exchange methods for producing high rydberg states, one group (R. F. Stebbings *et al.*, Rice University, Houston) reported that they had used tunable dye lasers in selecting individual high rydberg states in Xe and then measured the ionisation cross section in SF_6 . The use of these highly excited atoms in scattering experiments is now within the range of existing experimental techniques.

Lasers have not yet been exploited to the full in atomic physics for state selection. State and velocity selection of neutral atoms by photo-detachment of the negative ion (C. Linberger, GILA, Boulder, Colorado) is sure to advance the field of reactive scattering. Several groups are considering the determination of atomic beam composition by selective laser pumping.

Doppler free spectroscopy has advanced rapidly; fine and hyperfine transitions can now be measured more easily and accurately than before. The excitation of the $H(1S) \rightarrow H(2S)$ transition by two 2,341 Å photons, using the $H\beta$ level as a reference, has enabled the Lamb shift of $H(1S)$ to be measured. Multiphoton processes and level shifts using lasers are being extensively investigated in many laboratories in the USA and Europe.

The excellent electron scattering work done by many groups has now been complemented by positron scattering data. The measurement of positron differential scattering cross sections awaits the development of higher intensity sources.

The conference indicated very strongly the need now for experimental data on excited state phenomena, not only to aid applied research, but in order to understand better the nature of continuum interactions (U. Fano, University of Chicago) and the formation of bounding molecular states. □

Progress in binary circles

from John Faulkner

At three-year intervals since 1969, the International Astronomical Union has sponsored meetings to review some aspect of our knowledge of close binary stars. The latest and by general consent most successful meeting took place in and around the newly designated Hoyle building at the Cambridge Institute of Astronomy on July 28–August 1. Attendance has more than doubled at successive meetings; IAU Symposium No 73, "The Structure and Evolution of Close Binary Systems", attracted over 110 participants.

ONE reason for the rejuvenation of the subject has been the realisation that most galactic X-ray sources indeed consist of such systems in relatively short lived but highly observable stages of mass transfer between components. Herb Gursky (Harvard University), enjoying his role of Grand Old Man of X-ray astronomy, remarked on the new found respectability of his subject in being awarded the opening day rather than the last afternoon as before; he went on to show that while there is convincing statistical evidence that systems containing type II supernovae subsequently turn into X-ray sources, the situation is quite otherwise for type I. J. B. Hutchings (Dominion Astrophysical Observatory) reported an impressive spectroscopic confirmation of the disputed period of Sco X-1. Earlier this year Gottlieb, Wright and Liller (Harvard University) announced that a photometric study of old Harvard plates taken between 1889 and 1974 showed luminosity variations of only ~ 0.2 mag with a period of 0.787313 d. This remarkable discovery stood in serious conflict with post-X-ray discovery work by other groups and some scepticism had been expressed. However, Hutchings, Cowley and Crampton have now verified the Harvard period by studying the variable radial velocities of He II emission lines. Taken together, these investigations demonstrate the value of the classical and often lengthy programmes eschewed and frequently despised by the "black holier than thou" school of esoteric astrophysicists. Periods considerably less than a day (Cyg X-3 has $P \sim 4.8$ h) pose evolutionary problems for the picture of neutron stars orbiting more or less massive companions and both P. Ostriker (Princeton University)

and B. Paczynski (Polish Academy of Sciences, Warsaw) discussed how the systems might arise. Both required at an earlier stage that rapid mass transfer should produce a system with a neutron star orbiting the core of a distended giant star inside a common envelope. Ostriker envisaged an accretion wake to the neutron star inducing a gravitational drag on its orbit (reminiscent of Chandrasekhar's "dynamical friction"); concomitant heating blew away the envelope. Paczynski imagined the neutron star, through entrainment, acting as a giant paddle, transferring angular momentum from the motion to the common envelope. Both thought the system might at some stage resemble a planetary nebula surrounding a late-type star; whether these remarks were prompted solely by the presence of such puzzles in the literature was unclear. While obviously preliminary and exploratory, these suggestions stimulated much subsequent discussion.

B. Warner (University of Cape Town) presented an outstanding paper on observations of dwarf novae. While covering the many timescales exhibited by these objects, he emphasised the rapid periodicities ($P \sim 16\text{--}34$ s mainly) found by himself and colleagues. Warner and E. L. Robinson (University of Texas, Austin) had previously shown that the pulsations occurred only at or after outburst maxima, a result which everyone agreed placed severe constraints on outburst models (no-one being quite clear however just what the constraints were!) Warner now seems to have found one case of pulsation on the rising branch; it is questionable whether this will be the precursor of similar discoveries or the one exception that seems to exist to every generalisation in this field. Warner announced the discovery of three new types of periodicity in these variables (including one which, as Virginia Trimble remarked, is called "drifting subpulses" in pulsars), thus retaining for these systems the record for the largest number of interesting Fourier components. Almost everyone present now seems prepared to jump on the bandwagon of accretion disk instabilities to explain the quasi-periodic outbursts of these stars; the sceptic might remark that the defects of this explanation have yet to be as thoroughly explored as those of competing mechanisms.

Martin Rees (Cambridge University) gave a typically complete and ultimately sceptical review of accretion disk lore largely in the context of compact X-ray sources, ending with at least six criticisms of generally accepted assumptions as he ran out of time. X-ray emission processes, mass transfer dynamics, evolution and possible

tests of gravitational theories were all crying for attention and getting it in this trendy subject.

The classical topic of contact binaries provided the opportunity for two young workers to impress the audience with their computational skill in following cooperative (or even sometimes uncooperative) evolution of two stars. Both R. Webbink (Cambridge University) and B. Flannery (Institute of Advanced Study, Princeton) have developed modified forms of the evolutionary code of P. P. Eggleton whose method seems uniquely suited to this kind of problem. Webbink studied unsteady mass transfer in close binaries and produced an example in which a catastrophic runaway developed. Flannery showed how contact stars of

almost equal mass evolved to a stage of essentially cyclic thermal instability and mass exchange with mass fraction oscillating between ~ 0.56 and ~ 0.62 in periods of $\sim 10^7$ yr. Such systems seem a very satisfactory explanation for a number of the WUMa "main sequence contact binaries".

All in all, the symposium was a fine blend of observational facts, theoretical explanations and sheer unbridled speculation (at one point Warner remarking that the quality of the arguments led him to conclude that the cow lowing at 2-s intervals in an adjacent field had a density of $\sim 10^6$ g cm $^{-3}$). Commissars Mitton and Whelan marshalled the resources of the Institute splendidly for a meeting that will long be remembered. \square

Comets may conceal chemicals

from John Gribbin

Two of the more speculative of recent suggestions about the place of comets in the Galaxy have now been investigated in some detail by Fred Whipple (*Ast. J.*, **80**, 525; 1975). The ideas are that enough material may be locked up in comets to have a significant effect on the evolving chemistry of the Galaxy, and that γ -ray bursts may be produced by the impact of comets on to neutron stars. The score now seems to stand at Whipple 1, comets 1, with the first possibility still tenable but the second speculation now ruled out of order.

The gamma-ray burst hypothesis always did look a trifle implausible and seemed to require the presence of interstellar comets, since the violent events in which neutron stars are believed to form would be highly likely to disrupt any bound system of comets possessed by the original star. Even though a few comets with hyperbolic orbits have been seen, Whipple's calculations show that for four candidates—the only ones, in a sample of 89 comets with well determined parameters—the chance of any one having arrived on such an orbit from interstellar space is less than 1 in 10^4 , so that for the whole "family" to be interstellar they would be running against odds of 1 in 10^{16} . It is far more likely that the orbits were slightly hyperbolic because "of velocities imparted to solar system comets by the perturbation of passing stars, because of nongravitational effects induced by comet rotation and jet action, because of chance observational errors, or because of systematic displacements of the centre of the observed coma from the direction of the nucleus." But even without wandering through interstellar space free from their parent stars, comets can

play a part in the chemical evolution of the Galaxy. As any galaxy ages, the proportion of metals (by which astronomers mean anything except hydrogen and helium) in its stars increases through nucleosynthesis. Newly forming stars in an old galaxy contain the debris from previous stellar explosions, and do not have the same composition as newly forming stars in a young galaxy. But according to some calculations (B. M. Tinsley and A. G. W. Cameron, *Astrophys. Space Sci.*, **31**, 31; 1974) the mean metal abundance of disk population stars has grown more slowly than expected on the seemingly reasonable assumption that some 2% of the mass from one generation of stars is recycled in the next generation of star formation. Comets could provide a sink for the missing heavy metals (if any really are missing, of course), but Tinsley and Cameron need so many comets that their mass would be 25% of the total mass of gas in the interstellar medium of our Galaxy today—about 1% of the mass locked up in stars.

Rather surprisingly, perhaps, Whipple is able to show that a great mass of comets—more than 1% of the solar mass—could be gravitationally bound to the Sun in a symmetric shell just beyond the radius of the periodic comets and remain completely undetected by present techniques. This provides a lot of leeway for those theorists who wish to, to invoke the presence of such comets to explain discrepancies between their theories and observation, but will not please the more spartan souls who prefer to have well-defined limits on the parameters of the real Universe and who take great pains to fit their models within those rigid limits.

review article

Opiate receptor in normal and drug altered brain function*

Solomon H. Snyder†

Recent biochemical identification of the receptor site for pharmacological actions of opiates helps elucidate how these drugs relieve pain, elicit euphoria and 'addiction', and provides simple, direct approaches to developing potentially non-addicting analgesics. The isolation of a morphine-like peptide which may be a central nervous system neurotransmitter sheds light on normal brain mechanisms regulating pain and emotion.

EXTRACTS of the poppy plant have been used since the days of the Homeric epics medically and recreationally to relieve pain, induce sleep, ease anxiety, for diarrhoea, heart failure and simply to promote a sense of well being. It has long been known that opiates can be addictive and a major goal of opiate research is to develop a non-addictive opiate analgesic. Although non-opiate pain killers such as aspirin are useful in some situations, only opiates seem to be effective in the treatment of severe and protracted pain. Besides such a practical goal, understanding how opiates act may elucidate fundamental questions in pharmacology. Opiates provide the classic paradigm for tolerance and physical dependence, which can occur to a variety of drugs such as alcohol and barbiturates.

What opiates might tell us about normal brain function is a question that prompts consideration of the opiate receptor itself. The fairly stringent chemical requirements for opiate activity, the fact that opiate effects are highly stereospecific and that some opiates, such as etorphine, are so potent that they act in doses even lower than LSD, all suggest that opiate actions must involve highly selective sites or "receptors" in the brain. The existence of opiate antagonists also favours the receptor concept. Opiate antagonists are drugs, closely related in structure to the corresponding opiate analgesics, which may have little or no analgesic or euphoric effect themselves but which can completely reverse action of opiate analgesics. It seems as if the antagonists occupy opiate receptor sites, doing nothing themselves, but preventing access of opiate analgesics. If specific opiate receptors exist in the brain, one would assume that they were created for some normally occurring substance. Indeed, researchers have identified a normally occurring morphine-like factor in the brain with high affinity for specific opiate receptor sites. The distributions of the morphine-like factor and the opiate receptor itself correspond in part to certain pain pathways in the brain,

suggesting that the morphine-like factor may be a neurotransmitter for pathways in the brain mediating pain and emotions. A detailed understanding of such systems might help elucidate some riddles of addiction.

Demonstrating the opiate receptor

The most direct way to identify receptor sites is to measure the binding of radioactive drugs to brain tissue. Like most chemicals, however, opiates bind to almost any biological or non-biological membrane so that nonspecific binding, not associated with the receptor, greatly exceeded any receptor binding in most early studies. Goldstein *et al.*¹ enunciated the criterion that pharmacologically relevant opiate receptor binding should, like opiate analgesic effects themselves, be stereospecific.

In our laboratory, we developed simple technical manoeuvres to amplify specific receptor binding and decrease nonspecific binding. Thus, if opiates bind with considerable affinity to their receptor sites, the use of small amounts of drug labelled to high specific radioactivity favours specific, as opposed to nonspecific, binding, whereas washing tissue thoroughly but rapidly after binding preferentially removes nonspecific binding. On this basis, specific opiate receptor binding has been demonstrated to brain and guinea pig intestine²⁻⁴.

But stereospecific binding itself is not a sufficient criterion for the pharmacologically relevant opiate receptor. Some glass filters⁵ and numerous acidic lipids which may or may not be related to the biological receptor^{7,8} bind opiates stereospecifically. To ensure that binding to brain membranes represents receptor interactions that operate *in vivo*, many drugs were shown to exhibit affinity for the opiate receptor which closely parallels analgesic potency, while non-opiate drugs have negligible affinity for the opiate receptor. Ideally, one should compare pharmacological potency and receptor binding in the same system, which is feasible because the ability of opiates to inhibit electrically induced contractions of the guinea pig intestine closely parallels analgesic activity^{9,10}. Affinity of both opiate agonists and antagonists for receptor binding sites is closely similar in brain and guinea pig intestine and pharmacological and binding potencies of many opiate agonists and antagonists in the same strips of guinea pig intestine are well correlated¹¹. Besides supporting the other evidence that binding *in vitro* involves sites that mediate opiate activity

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in vivo, these observations shed light on certain basic drug receptor concepts. Some receptor theories postulate that drugs can exert maximal pharmacological responses while occupying only a small fraction of the total number of pharmacologically active receptors. If a substantial portion of opiate receptors does not normally mediate pharmacological responses, and are thus "spare receptors", then there should be major discrepancies between drug concentrations filling a given percentage of binding sites and concentrations required to produce the same percentage of a maximal pharmacological response. Opiate concentrations occupying half the binding sites in the guinea pig intestine correspond closely, however, to those eliciting half-maximal pharmacological responses, whether agonist or antagonist, which suggests that opiate effects can be explained without invoking spare receptors.

Differential receptor interactions of opiate agonists and antagonists

Opiate antagonists are important for several practical and theoretical reasons. Pure opiate antagonists, such as naloxone and naltrexone, are often life-saving antidotes to opiate overdose. Pure antagonists may also be valuable in treating heroin addicts. The notion is to administer a long-lasting pure antagonist, which will produce no analgesia and euphoria of itself, but which will prevent the effects of subsequently administered heroin. Thus deprived of its ability to elicit a 'high', heroin will cease to be attractive to the addict.

Of greater theoretical and practical importance are opiates which are mixed agonist-antagonists, since some of these agents are relatively non-addicting analgesics. The idea of combining antagonist with agonist activity to lessen the addictive potential of an opiate stems from demonstrations that the opiate antagonist nalorphine possesses analgesic, and thus "agonist" properties¹². Unfortunately, nalorphine, cyclazocine, and levallorphan, antagonists 'contaminated' with agonist activity, are not suitable for routine use in treatment of pain, because at analgesic doses they often produce anxiety, agitation, and psychotomimetic effects. Antagonists with yet more agonist activity, referred to here as mixed agonist-antagonists, have been more promising. Pentazocine, a mixed agonist-antagonist, is already clinically marketed and relieves pain with a low incidence of psychotomimetic effects and with relatively little propensity to produce physical dependence and compulsive craving. Several other mixed agonist-antagonists, while not yet in routine clinical use, offer promise as relatively non-addicting analgesics.

Traditional opiate antagonists differ chemically from agonists only in the substitution of an N-allyl, N-cyclopropyl or related group for the N-methyl of agonists. It is quite difficult to predict chemical features which result in a mixed agonist-antagonist. In our early studies of opiate receptor binding with buffer solutions lacking sodium, agonists and antagonists bound with similar affinity³. Later, we discovered that low concentrations of sodium enhance receptor binding of antagonists but decrease the binding of agonists^{13,14}. The effect of sodium is highly specific. It can be mimicked to some extent by lithium, whose atomic radius is similar to that of sodium, but not by other monovalent cations such as potassium, rubidium or caesium. Sodium seems to accelerate the rate at which antagonists bind to the receptor¹⁵ and to speed the dissociation of agonists from the receptor¹⁴. To evaluate the influence of sodium on receptor interactions of a wide range of opiate agonists and antagonists, we measured the extent to which sodium alters the ability of drugs to inhibit receptor binding of ³H-naloxone. The resultant "sodium response ratio" is the ratio of the concentration of drug which inhibits ³H-naloxone binding 50% in the presence of sodium to the

comparable concentration when sodium is omitted from the incubation medium. This sodium response ratio differentiates opiate agonists and antagonists. For pure antagonists, such as naloxone, the ratio is 1, while for a variety of opiate agonists, the ratio is between 12 and 60. Antagonists which are 'contaminated' with some agonist activity have ratios somewhat greater than 1 but less than 3. The mixed agonist-antagonists we examined have ratios between 3.3 and 6.4, clearly in an intermediate area.

Thus it seems possible to predict the pharmacological properties of different opiates simply by measuring receptor interactions in the presence and absence of sodium, affording a rapid and inexpensive screen for mixed agonist-antagonist 'non-addicting' analgesics. Chemists need not synthesise hundreds of grams of drug for screening in intact animals; a fraction of a milligram will suffice.

Model of opiate receptor function

The selective and pharmacologically relevant influence of sodium on opiate receptor binding seems an integral feature of receptor functioning. Sodium seems to increase and decrease respectively the numbers of antagonist and agonist receptors. Since opiate agonists and antagonists are so similar chemically and compete with each other for receptor binding, we postulate that both drugs bind to the same receptor but that the opiate binding site can vary as the receptor is transformed between two conformations^{14,16,17}. With normal body sodium levels, the receptor is largely in the "sodium" state for which antagonists have a selectively high affinity, whereas opiate agonist actions take place when agonists bind with their selectively high affinity for the "no-sodium" state. Antagonists block morphine actions by occupying sodium states of the receptor and shifting the equilibrium to reduce the number of no-sodium receptors available for morphine. Although agonists and antagonists have selective high affinity for no-sodium and sodium states of the receptor respectively, mixed agonist-antagonists would have similar affinities for the two forms.

The interconversion of the two forms of the opiate receptor presumably involves folding, unfolding, aggregation, disaggregation or other modifications in protein structure, since the exquisite sensitivity of receptor binding to proteolytic enzymes¹⁸ indicates that the opiate receptor is proteinaceous. One might expect protein-modifying reagents and enzymes that affect protein structure to alter the interconversion of receptor sites. Since the opiate receptor should normally be largely in the antagonist or sodium state, agents interfering with receptor interconversion should selectively reduce agonist binding. Consistent with these predictions is the observation that protein-modifying reagents and proteolytic enzymes in low concentrations do selectively reduce opiate agonist binding with negligible effects on antagonist binding¹⁹⁻²¹. The effects of protein-modifying reagents and enzymes seem to be directly related to sodium binding by the opiate receptor, for the reagents and enzymes increase the sensitivity of opiate agonist binding to inhibition by sodium.

Divalent cations may also have a role in the normal functioning of the opiate receptor. Low, physiological, concentrations of manganese and magnesium affect the opiate receptor in a fashion diametrically opposite to sodium; they selectively increase the binding of opiate agonists, by reducing receptor sensitivity to sodium²². Divalent cations act selectively; for while manganese, magnesium and nickel are active, calcium fails to enhance opiate agonist binding. The influence of chelating agents indicates that endogenous divalent cations regulate opiate receptor functions. Thus, treating brain membranes with EDTA, which chelates most divalent cations, lowers opiate agonist binding, while EGTA, which chelates calcium but not manganese and magnesium, has no influence on receptor binding.

The postulated interconversion of agonist and antagonist

conformations and the influence of sodium resemble general models for neurotransmitter receptor behaviour²²⁻²⁵. Normally receptors sites are in the antagonist or "off" conformation. Synaptic transmission occurs only when a compound binds selectively to the agonist conformation of the receptor. One or the other of the receptor states possesses a binding site for the ion whose conductance is altered in synaptic transmission. When the neurotransmitter attaches to the receptor, the binding of this ion is altered to produce the appropriate change in ionic conductance. If the endogenous morphine-like substance is a neurotransmitter, one would speculate that sodium is the ion whose conductance is changed by synaptic activity.

Direct evidence for selective interactions of an ion and receptor binding in synaptic transmission has been obtained for the influence of chloride on glycine receptor binding. Receptor sites for glycine, a major inhibitory neurotransmitter in the mammalian central nervous system, can be labelled with radioactive strychnine, a potent glycine antagonist²⁶. Chloride is the ion whose conductance is increased by the inhibitory synaptic actions of glycine. Chloride and other anions inhibit the binding of strychnine to the glycine receptor in proportion to their ability to mimic the synaptic activities of chloride²⁷.

Regional mapping

Brain functions whose distributions parallel those of opiate receptors may be implicated in opiate actions. Because opiates elicit analgesia, brain structures mediating pain are natural suspects. Sharp, prickly pain, which can be discretely localised, is conveyed by a laterally located sensory pathway with somatotopic representation in the ventrobasal thalamus among other regions. Slower, more chronic and poorly localised burning pain involves multisynaptic, slowly conducting, medially located pathways, especially the palaeospinothalamic and spinoreticulodiencephalic pathways. These lack somatotopic representation and include areas of the brain such as the periaqueductal grey, the medial nuclei of the thalamus and several parts of the limbic system²⁸.

Opiate receptor binding varies dramatically throughout the monkey and human brain^{29,30}. The amygdala binds most, but only slightly more than the periaqueductal grey of the midbrain, hypothalamus and medial thalamus. Receptor binding in the caudate nucleus is also high. Within the cerebral cortex there are marked variations: the frontal cortex for example binds more than four times the amount of opiate bound by the precentral, postcentral gyri and occipital pole. Receptor binding is very low in the spinal cord, cerebellum and white matter.

This map closely resembles the distribution of palaeospinothalamic and spinoreticulodiencephalic pain pathways, and certain of these areas, especially the periaqueductal grey, correspond to those in which implantation of morphine most effectively produces analgesia³¹. Opiate antagonists elicit withdrawal symptoms in addicted animals best when implanted in the medial thalamus, which is also very rich in opiate receptors³². The amygdala, apparently an exception not classically associated with pain pathways, may relate to affective components of pain since electrical stimulation of the amygdala does produce fight and flight reactions. Euphoric actions of morphine may be mediated by the amygdala and other areas of the emotion-regulating limbic system rich in opiate receptors.

Recent autoradiographic studies provide even more precise localisation of opiate receptor sites³³. For such studies it was first necessary for us to achieve reliable techniques for labelling receptors in the intact animals³⁴. After intravenous administration of ³H-diprenorphine, an extremely potent antagonist, 80% or more of radioactivity accumulating in the brain is associated with opiate receptor sites³⁵. For successful autoradiography, diffusion of the radioactive drug away from the receptor site during the fixation

process must be prevented, a difficult task with a drug such as an opiate which does not form covalent chemical linkages to tissue proteins during fixation. In our studies, fixing tissue at very low temperature prevents leakage of ³H-diprenorphine from receptor sites so that essentially all autoradiographic grains are associated with the opiate receptor³³. Grain density parallels the regional distribution of the opiate receptor as determined by dissection and biochemical assays. At a microscopic level, opiate receptor binding is even more discretely localised (Table 1). At one level of the midbrain, grains are highly concentrated over the locus coeruleus with much lower grain counts in closely adjacent nuclei. Interestingly, small doses of morphine slow firing rates of locus coeruleus cells but not adjacent cells³⁵. Since the locus coeruleus consists almost exclusively of noradrenaline cell bodies, its high density of opiate receptors may explain the numerous reports of effects of opiates on the metabolism of biogenic amines including noradrenaline³⁶. At another level of the midbrain, grains concentrate over the zona compacta of the substantia nigra, whereas the adjacent zona reticulata of the substantia nigra has very few receptor grains. The zona compacta but not the zona reticulata consists exclusively of dopamine cell bodies. Thus both major catecholamine systems on the brain are intimately related to opiate receptor sites. An association with major sensory systems in the central nervous system is indicated by the sharp band of opiate receptor grains overlying the substantia gelatinosa in the lower brain stem and spinal cord³³. The substantia gelatinosa is an important "way station" for the upward conduction of sensory information relating to pain.

Table 1 Distribution of autoradiographic grains labelling the opiate receptor in coronal sections of rat brain

Region	Autoradiographic grains per 100 μm^2
Nucleus caudatus putamen	
"Streak" ventral to corpus callosum	11.5
"Clusters"	10.5
Low density areas	1.7
Fibres	1.2
Substantia gelatinosa (spinal cord)	10.4
Locus coeruleus	10.0
Amygdala medialis	6.5
Amygdala centralis	5.8
Zona compacta of substantia nigra	6.5
Thalamus medialis	5.5
Periventricular substance	5.2
Habenula	5.1
Lateral habenula	5.0
Nucleus periventricularis (of hypothalamus)	3.8
Nucleus ventromedialis (of hypothalamus)	2.8
Dentate gyrus	2.1
Motor cortex	2.0
Zona reticulata of substantia nigra	1.9
Hippocampus	1.7
Corpus callosum	0.7
Fimbria	0.8
Ventricle III	1.3
Pyriform cortex	1.1
Cerebellum	0.8
Nucleus of cranial nerve V	0.8

These data are derived from the study of Pert, Kuhar and Snyder³³. Rats (170 g) were injected with 125 μCi of ³H-diprenorphine (13 Ci mmol^{-1}), killed at 1 h and brains rapidly cut into 3-4 mm coronal sections, placed on microtome chucks and lowered slowly into liquid nitrogen "slush". Sections of 4 μm thickness were cut at -18°C in a Harris cryostat microtome and transferred by "thaw-mounting" in the dark to slides already coated with Kodak NTB-3 emulsion. After 5 weeks of exposure at low humidity (4°C) in a dark lead-lined cabinet, slides were developed at 17°C in Dektol for 2 min. Development was terminated by Kodak Liquid Hardener and slides were fixed in hypo (Kodak). After washing in running tapwater, sections were stained with pyronine Y, dried, mounted with Permount and viewed with a Zeiss Universal microscope. Control slides prepared for positive and negative chemography showed no significant fading of latent images or spurious generation of grains after 60 d of exposure. Grain counts are the means of 2,400 μm^2 areas.

Table 2 Regional localisation of the morphine-like factor and opiate receptors in bovine and rat brain

	Morphine-like factor (U per mg protein)	Opiate receptor binding (c.p.m. per 0.1 mg protein)
Bovine		
Caudate	480	320
Hypothalamus	250	282
Spinal cord	140	205
Pons	135	231
Cerebral cortex (Parietal)	80	173
Thalamus	75	179
Cerebellum	50	86
Medulla oblongata	50	88
Corpus callosum	10	61
Rat		
Caudate	480	900
Brainstem (midbrain)	140	220
Cerebral cortex	80	210
Cerebellum	None	None

Data are derived from the study of Pasternak and Snyder⁴¹. In assays for the morphine-like factor, brains were homogenised in ten volumes 0.32 M sucrose with a Potter-Elvehjem homogeniser, and centrifuged at 100,000g for 1 h. The pellet was resuspended in 2 volumes of 10 mM Tris-HCl buffer (pH 7.7 at 25 °C) immersed in a bath of boiling water for 15 min, recentrifuged at 100,000g for 1 h and the supernatant assayed by adding 50–200 µl to an opiate receptor binding assay with ³H-naloxone and 1 mM MnCl₂. Opiate receptor assays were performed as previously described²⁰. The experiments were replicated four times with less than 20% variation between experiments.

Mapping opiate receptor sites by autoradiography is still in a preliminary stage. It is however already clear that this information will help greatly in elucidating the physiological and pharmacological roles of opiate receptor sites. Neurophysiologists who would like to record from cells containing opiate receptor sites ought to focus on the locus coeruleus, the zona compacta of the substantia nigra and other areas enriched in these receptors.

The endogenous morphine-like factor

Man was not made with morphine in him. Is the opiate receptor 'designed' for a normally occurring morphine-like substance, perhaps a neurotransmitter? If so, such a system is widespread among animals. Opiate receptor binding with almost identical substrate specificity has been detected in the central nervous system of all vertebrates, and the most primitive fish have as many opiate receptors as mammals including man³⁷. Strikingly, no opiate receptor binding can be demonstrated in any invertebrates, which may indicate a relationship of the opiate receptor to known differences in patterns of vertebrate and invertebrate neuronal connections. The possibility that the opiate receptor is associated with a neurotransmitter system is strengthened by subcellular fractionation studies which indicate that in mammalian brain homogenates opiate receptor binding is most enriched in synaptosome fractions containing nerve endings with their associated postsynaptic membranes³⁸. When these are subjected to hypotonic lysis, opiate receptor binding is recovered primarily in the synaptic membrane fraction, as would be expected for a neurotransmitter receptor³⁸. A direct approach to identifying a possible neurotransmitter associated with the opiate receptor was adopted by Hughes³⁹, who found in brain extracts a substance which mimics the ability of morphine to inhibit electrically induced contractions of smooth muscle preparations such as the mouse vas deferens or guinea pig intestine, while Terenius⁴⁰ and Pasternak and Snyder⁴¹ reported that brain extracts compete for opiate receptor binding. The substance studied

by both approaches seems to be the same or very similar. The morphine-like factor or "enkephalin" is a peptide, degraded by carboxypeptidase A and B as well as leucine-aminopeptidase and to a lesser extent by chymotrypsin but not by trypsin^{39,41,42}. In opiate binding assays, the material behaves like an agonist, since its ability to compete for binding is impaired by sodium and protein modifying reagents but enhanced by manganese⁴¹. Its regional distribution in calf, rat and rabbit brain is closely similar to that of the opiate receptor itself (Table 2)³⁸⁻⁴². Preliminary chemical analysis indicates that the morphine-like factor contains three residues of glycine and one each of phenylalanine, tyrosine and methionine and may also contain tryptophan^{39,42}. A chemically different peptide, which also mimics the effect of morphine on smooth muscle, has been isolated from pituitary glands⁴³.

If the morphine-like factor is a neurotransmitter, how might it affect postsynaptic cells? Morphine and other opiates applied iontophoretically to single cerebral cortex cells inhibit firing in proportion to their pharmacological potency with marked stereospecific actions and antagonism by naloxone, suggesting that the morphine-like factor may be an inhibitory transmitter⁴⁴. Cells of rats addicted to morphine are no longer inhibited by administered morphine and may even show a paradoxical excitation. Because these changes with addiction occur at the opiate responsive cell itself, the locus of the fundamental alteration in opiate addiction would seem to be at the level of the cell membrane possessing the opiate receptor.

Possible mechanisms of addiction

The term "addiction" is not easily defined. Most people consider opiate addiction to comprise three major elements: tolerance, physical dependence, and compulsive craving. Tolerance means simply that, after prolonged administration of a drug, the organism can "tolerate" more of it, that is, it is now less sensitive to the drug than before. Physical dependence refers to the fact that when the drug is withdrawn or when the animal or person is treated with an opiate antagonist, withdrawal symptoms become evident which are usually in a direction opposite to the initial effects of the drug. Thus whereas morphine produces pupillary constriction, constipation and sedation, during withdrawal the pupils are dilated and there is diarrhoea and central excitation. Compulsive craving is very difficult to evaluate; it refers to the addict's propensity for the drug even long periods after he has been physically withdrawn. Just as tolerance can be looked on as a state of decreased sensitivity to opiate agonists, so in physical dependence animals and humans become more sensitive to antagonists. Withdrawal symptoms can be elicited by much lower doses of antagonists in severely than in mildly addicted animals.

There are two types of model which explain opiate addiction; one of them involves a change at the level of the opiate receptor or closely allied structures, whereas the other requires no such alterations⁴⁵⁻⁴⁹. In the latter model, one presumes that morphine suppresses some neuronal system in the brain. To compensate, a completely separate system increases its activity to counteract the suppressive action of morphine; whereupon the organism is "tolerant". When morphine is withdrawn, the "overactive" second system produces withdrawal symptoms.

Hypersensitivity to antagonists coupled with subsensitivity to agonists in addiction is reminiscent of the properties of the sodium or "antagonist" state of the opiate receptor as contrasted to the no-sodium or "agonist" state. It may be speculated that tolerance and physical dependence reflect a change in the opiate receptor so that it is less capable of assuming the agonist form, favouring instead the antagonist, sodium form. If this were so, addicted animals should be "subsensitive" to opiate agonists, since the receptor would

be less frequently in the "agonist" state to mediate drug effects. Similarly, the organism would be supersensitive to antagonists because of the predominant sodium or antagonist state of the receptor. Though opiate receptor assays both *in vitro* and *in vivo* have failed to show systematic changes related to the addicted state^{13,50}, it is conceivable that such a subtle alteration may be reflected only in membrane properties which occur secondarily to the binding itself. In this connection, recent studies of opiate effects on a neuroblastoma-glioma hybrid in cell culture are illuminating. Opiate agonists, in proportion to their affinity for opiate receptor binding sites in this clone, decrease adenylate cyclase^{51,52} and enhance the accumulation of cyclic GMP⁵³. Opiates also reverse the stimulation of adenylate cyclase by prostaglandin E₁ and adenine⁵¹. Cells exposed chronically to morphine become "tolerant" to the effects of morphine so that previously active doses no longer antagonise the prostaglandin stimulation of adenylate cyclase⁵⁴. When the cells are put into "withdrawal" by naloxone, they become "supersensitive" to the ability of prostaglandin E₁ to stimulate adenylate cyclase, an effect opposite to that of morphine itself in drug-naïve cells⁵⁴.

Of course, one might argue that the cyclic nucleotide changes are relevant only to these cancerous cells of the nervous system in tissue culture. Previously, Collier and Roy⁵⁵ had described a prostaglandin-stimulated adenylate cyclase in mammalian brain which is inhibited by morphine and other opiates, and may be related to the neuroblastoma-glioma effects. Moreover phosphodiesterase inhibitors, which elevate brain cyclic AMP levels, elicit in rats behavioural changes resembling the opiate withdrawal syndrome and which are enhanced by as little as 0.03 mg kg⁻¹ of naloxone^{56,57}.

In summary, recent identification of opiate receptor sites in the central nervous system by biochemical means has spurred a great body of research directed both at pharmacological actions of opiates as well as at the normal functioning of the opiate receptor. An apparently novel peptide neurotransmitter, the morphine-like factor, is being characterised.

As in the case with numerous hormones and neurotransmitters, this morphine-like factor may have as its second messenger cyclic nucleotides, either cyclic AMP or cyclic GMP. Most dramatically, it seems possible that changes in the adenylate cyclase associated with the opiate receptor and conceivably in the opiate receptor itself may begin to answer the many riddles of addiction.

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articles

Quark charges and nuclear β -decay

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The rate of β -decay is affected by the charges, Q , of the nucleon's constituent quarks. The Salam-Weinberg scheme permits us, in a spirit of adventurous naivety, to conclude that $\bar{Q} = 0.20 \pm 0.10$; this combines with electron and neutrino data to suggest individual quark charges: $Q_p = 0.69 \pm 0.07$, $Q_n = -0.29 \pm 0.15$.

The quark-parton model

The highly successful quark model for the description of hadronic spectra^{1,2} considers baryons as made up of three quarks of baryon number 1/3 and of charges that, in the classical version of the model, are $Q_u = 2/3$ and $Q_d = -1/3$ times the electronic unit but that, in alternative versions, may be integral. The parton model of the nucleon, originally inspired by the scaling

phenomena of deep inelastic electron scattering and by high energy hadronic phenomena^{3,4}, considers the nucleon to be made of "point-like" subunits—the partons. Identification of quarks with partons gives the quark-parton model.

The literal parton model is encouraged by several phenomena such as:

- (1) the surprisingly large abundance of particles produced with high transverse momentum in pp collisions at high energy such as the 28+28 GeV experiments at the CERN ISR⁵;
- (2) the linearity of neutrino-nucleon total cross section with neutrino energy up to $E \simeq 200$ GeV (ref. 6); the latter data indicate that the total effective dimension of the νN interaction, including that of the parton, is less than 0.02 fm which, on comparison with $\langle r^2 \rangle^{1/2} \simeq 0.8$ fm for the nucleon as a whole, suggests that the partons are, at the most, 2–3% of the size of the nucleon that they make up.

That the spin of the partons is $\frac{1}{2}$ follows experimentally from the deep inelastic electron scattering experiments (the Callen-Gross relation)⁶; the experimental ratio of 3:1 between high energy neutrino and antineutrino cross sections on nucleons then shows that the nucleon is composed 95% or more of partons and therefore has very little antiparton content⁶ (some antimatter there must be since forces must make pairs). The Gross-Llewellyn-Smith sum rule for neutrino-nucleon interactions permits the "counting" of the partons; the result of 3.2 ± 0.6 completes the "experimental" identification of the quark-parton model⁶.

Of course, one is not obliged to talk this language at all. One can replace the literal constituent quarks by current quarks and one can replace the literal point-like partons by statements about singularities of relevant commutators on the lightcone. But the quark-parton model provides, at least, a legitimate, and attractively picturesque, framework for the parameterisation of particle structure and, within that framework, it may be asked whether an internal consistency with one or other version of the model is found. In this article I adopt the simple quark-parton viewpoint, ignore its naiveties and illuminate it through ordinary low energy nuclear β -decay.

The essential point is that β -decay does not involve solely the weak interaction; electromagnetic forces must be simultaneously at work providing the "radiative corrections". The radiative corrections involve not only the nucleon as a whole but also its constituent quarks so the magnitude of the radiative corrections must involve the charges of the quarks; this article makes statements about those charges.

We proceed through a hierarchy of hypotheses: (1) conservation of the vector current⁷; (2) μ -e universality in the Cabibbo sense⁸; (3) unification of the weak and electromagnetic interactions in the Salam-Weinberg sense⁹. Without (1) and (2) the present remarks fall to the ground. Adoption of (3) makes our evaluation specific but it could be redone for any other version of the gauge theories than Salam-Weinberg and any other version of the quark model than those used by Sirlin¹⁰ in his contingent treatment of the radiative corrections. In view of the uncertainties attaching to (3) the present analysis should perhaps be regarded as no more than illustrative of the sort of information as to the quark charges that will ultimately be extracted from nuclear β -decay; but if, as seems possible, Sirlin's result for the radiative corrections has a rather general validity, we may already regard the present outcome as of interest. We find:

$$\bar{Q} = 0.20 \pm 0.10$$

where \bar{Q} is the mean quark charge of the nucleon (compare $1/6$ expected for the classical non-integrally-charged quark model^{11,2} and $1/2$ for integrally-charged quark models).

The radiative corrections

Briefly, it is clear^{11,12} that the radiative corrections of order α to nucleon β -decay may be divided into two parts: the well-understood explicit "outer" correction δ_B^a is energy-release dependent but independent of nucleon structure and β -decay anatomy

and may therefore be absorbed into the phase-space factor; the "inner" correction Δ_B^a depends on structure and anatomy but is energy-release independent so may be absorbed into the coupling constant. We particularise to vector β -decay and write:

$$g_{\beta V}^R = g_{\beta V}(1 + \frac{1}{2}\Delta_{\beta V}^a)$$

where $g_{\beta V}$ is the vector coupling constant as we should know it but for the radiative corrections.

Our hope of quark-charge information therefore resides in $\Delta_{\beta V}^a$. The trouble about $\Delta_{\beta V}^a$ used to be the divergencies that it contained: these were severe and largely intractable, even when the primitive point 4-fermion interaction $N \rightarrow N\bar{\nu}e$ (ref. 13) was replaced by mediation through the hypothetical intermediate vector boson $N \rightarrow N W; W \rightarrow e\bar{\nu}$ (ref. 14) and when quark structure was introduced¹⁵. The new unified weak/electromagnetic gauge theories⁹ have, however, changed all that: $\Delta_{\beta V}^a$ is now divergenceless apart from a single term that can, unexceptionably, be absorbed into the weak coupling constant as a universal renormalisation in direct analogy to the hallowed practice in quantum electrodynamics.

$\Delta_{\beta V}^a$ must be evaluated in the context of specific quark models and specific formulations of the unified theory; so far only two classes of quark model have been studied in detail¹⁰ and one formulation of weak interaction theory, namely the Salam-Weinberg scheme⁹; however, the general form of $\Delta_{\beta V}^a$ has remained remarkably stable against changes in its conceptual background^{10,13-15} and it seems probable that something very like the present form will remain no matter what the quark and weak interaction pictures maybe come. We therefore conduct our present analysis using Sirlin's¹⁰ evaluation of $\Delta_{\beta V}^a$, recognising that the Salam-Weinberg scheme on which it is based is by no means proven and recognising the limited range of quark pictures for which it has been shown to be valid but believing that something very like it will continue to hold and therefore regarding the conclusions that we tentatively draw here as being, at the least, illustrative of the quality of information that can, in principle, be extracted from β -decay data.

We can choose whether we want to use β -decay to tell us about nucleon structure or about the anatomy of β -decay. The earlier viewpoint, pioneered by Blin-Stoyle, to whom a great deal is due in studies of the present type¹⁶, was that β -decay suggested mediation of the weak interaction by a very heavy intermediate vector boson; the success of the gauge theories now seems to recommend that we tentatively take the weak interaction for granted and use β -decay to tell us about nucleon structure; this is the viewpoint of my present note.

What is of interest is not so much $\Delta_{\beta V}^a$ itself as $\Delta_{\beta V}^a - \Delta_\mu^a$ where Δ_μ^a is the analogous quantity involved in muon decay. Sirlin¹⁰ finds

$$\Delta_{\beta V}^a - \Delta_\mu^a = (\alpha/2\pi) \{3\ln(m_Z/m_N) + 6\bar{Q}\ln(m_Z/m_A)\}$$

The first term in curly brackets comes from the vector part of the hadronic weak current; the second term comes from the axial part of the hadronic weak current which contributes to the overall vector amplitude because it can combine appropriately with the isoscalar part of the electromagnetic current. m_N is the nucleon mass and m_Z that of the neutral intermediate vector boson, Z^0 , of the Salam-Weinberg scheme (the mass of the charged W^\pm drops out in $\Delta_{\beta V}^a - \Delta_\mu^a$). m_A is the mass of some suitable "axial" structure and is conventionally awarded the mass of the A_1 -meson for which we take 1,300 MeV (ref. 17 and M. G. Bowler, private communication); this is the energy above which the asymptotic form for the axial current part of the second rank tensor that describes the exchange of virtual photons between nucleon and decay electron becomes valid. m_A is uncertain but it would have to be raised to 4.5 GeV to shift our present conclusion as to \bar{Q} by its error limit.

When we are concerned with nuclear, as opposed to nucleon,

β -decay "outer" radiative corrections arise in addition to δ_β^a . If one writes them as δ_β^{za} a general restriction is $m \geq n$ (ref. 18). By the Ademollo-Gatto theorem¹⁹, which says that the renormalisation of coupling constants is of second order in the multiplet mass splittings, there is no term linear in $Z\alpha$ (ref. 18). Higher terms with $m = n$ and not involving nuclear excitation are negligible²⁰; those involving nuclear excitation are treated

such as has been occasionally suggested as an explanation for the anomalously high rate of $\eta \rightarrow \pi$ decay, would have negligible effect²³).

For such $J^\pi = 0^+$, $T = 1$ decays, including all radiative corrections, we can therefore write:

$$f^R t = \frac{\pi^3 \hbar^7 \ln 2}{m^6 c^4 g_{\beta V}^2 (1 - \epsilon)} = \frac{6.153 \times 10^{-25}}{g_{\beta V}^2 (1 - \epsilon)} \text{ erg}^2 \text{ cm}^6 \text{ s}$$

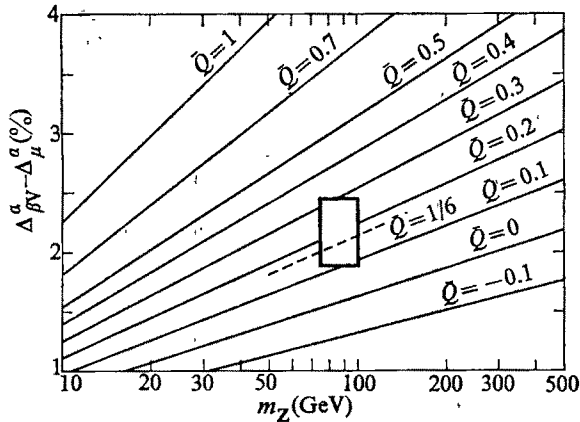


Fig. 1

separately as small modifications to the nuclear matrix element as I shall discuss in detail. Of the terms with $m = n+1$, $\delta_\beta^{za^2}$ and $\delta_\beta^{za^3}$ have been evaluated^{21,22} and the remainder shown to be negligible (for the nuclei of present concern). Terms with $m = n+2$ and so on are also negligible^{21,22}; we therefore, with an overall accuracy of better than 0.1%, replace the ordinary phase space factor f (which already contains the effect of the gross Coulomb field of the nucleus in distorting the outgoing electron wave) by its outer-radiative-corrected version f^R :

$$f^R = f(1 + \delta_\beta^a + \delta_\beta^{za^2} + \delta_\beta^{za^3})$$

(The δ_β terms are designed to be combined in this way)

We remember that other radiative effects are contained in $g_{\beta V}^R$ and in the modification of the nuclear matrix element.

Pure vector nuclear β -decay

β -decay within isospin multiplets of $J^\pi = 0^+$ is pure vector because there is no nuclear spin to flip. Under complete charge independence the matrix element would be given purely by the geometrical Clebsch-Gordan factor in the isospins and, for the cases of $T = 1$ with which we are here concerned, would take the value $\sqrt{2}$. In practice, charge independence is relaxed by the implicit and explicit effects of the Coulomb force so that the matrix element must be multiplied by $(1 - \epsilon)^{1/2}$ where we expect, by the Ademollo-Gatto theorem, that ϵ should go roughly as Z^2 . (Non-Coulomb breaking of charge independence,

where t is the half life (corrected for branching and orbital electron capture) and where there is no error in the numerical value to the accuracy stated.

f itself must be computed with care and must involve solving the Dirac equation in a field generated by a nuclear charge distribution of finite size, consistent with electron scattering and muonic X-ray data, screened by the atomic electrons, and convoluting the resultant positron and associated neutrino wavefunctions with nucleonic wavefunctions generated in a realistic nuclear potential. This task has been performed with an overall reliability of better than 0.05% (refs 24 and 25).

The ϵ have been computed several times recently and their associated error is probably better than 0.1%; this matrix element correction can also be derived empirically from the experimental data (see section under that heading), as reviewed in the section headed ϵ -values, when it shows good agreement with the directly-computed values.

The experimental data relate to the decay of ^{14}O , $^{26}\text{Al}^m$, ^{34}Cl , $^{38}\text{K}^m$, ^{42}Sc , ^{46}V , ^{50}Mn and ^{54}Co for each of which bodies ft is determined to better than $\pm 1/3\%$. The set shows excellent internal consistency and we conclude

$$g_{\beta V}^R = (1.4116 \pm 0.0008) \times 10^{-49} \text{ erg cm}^3$$

Conserved vector current and the Cabibbo angle

The excellent internal consistency of the eight accurately-measured vector transitions agrees with the hypothesis of the conserved vector current⁷ in that vector β -decay suffers no renormalisation by mesonic exchange effects and may therefore be equated with muon decay if we extend the hypothesis to a universality of the weak coupling constant in analogy to that of electric charge. In adopting the hypothesis of universality we must follow Cabibbo⁸ and divide the hadronic weak current into strangeness-conserving and strangeness-changing parts so that our expectation becomes:

$$g_{\beta V} = g_\mu \cos \theta_C$$

where θ_C is the Cabibbo angle that splits the hadronic decay strength between $\Delta S = 0$ and $\Delta S = 1$.

This expectation is not vitiated by W-boson propagator corrections which are of order $(m_\mu/m_W)^2$ or less, that is less than 0.01% for $m_W > 10 \text{ GeV}$ as experimentally demonstrated⁶, nor by finite nucleon size effects which amount to (2/21)

Table 1

Decaying body	$E_0(\text{keV})$	$t(\text{s})$	$f^R t(\text{s})$	$f t \text{ s}$
^{14}O	$1,809.86 \pm 0.36$ (ref. 24)	71.136 ± 0.031 (ref. 24)*	$3,094.3 \pm 3.0$	$3,090.3 \pm 4.3$
$^{26}\text{Al}^m$	$3,210.7 \pm 0.5$ (ref. 36)	6.352 ± 0.005 (ref. 24)	$3,085.9 \pm 3.3$	$3,080.3 \pm 4.5$
^{34}Cl	$4,467.9 \pm 1.3$ (ref. 36)	1.531 ± 0.004 (ref. 36)	$3,100.7 \pm 9.0$	$3,088.0 \pm 9.5$
$^{38}\text{K}^m$	$5,021.2 \pm 3.4$ (ref. 37)	0.9264 ± 0.0007 (ref. 37)	$3,103.4 \pm 9.9$	$3,091.9 \pm 10.4$
^{42}Sc	$5,399.9 \pm 2.2$ (ref. 36)	0.6846 ± 0.0009 (ref. 24)	$3,104.7 \pm 7.1$	$3,092.3 \pm 7.8$
^{46}V	$6,018.8 \pm 2.8$ (ref. 36)	0.4258 ± 0.0007 (ref. 38)	$3,099.1 \pm 8.4$	$3,090.1 \pm 9.0$
^{50}Mn	$6,607.8 \pm 2.1$ (ref. 36)	0.2837 ± 0.0006 (refs 38, 39)	$3,097.0 \pm 8.0$	$3,086.8 \pm 8.6$
^{54}Co	$7,219.0 \pm 1.6$ (refs 36, 40)	0.1934 ± 0.0003 (refs 38, 40)	$3,102.9 \pm 5.3$	$3,090.8 \pm 6.1$

* And J. A. Becker, R. L. Chalmers, B. A. Watson, and D. H. Wilkinson, unpublished.

$W_0^2 \langle r^2 \rangle_{\beta\gamma}$, where W_0 is the energy release in natural units, 0.01% in the worst of our present cases.

The Cabibbo angle is most naturally determined from semi-leptonic hyperon decay on which a large and excellently self-consistent body of data exists²⁶ and which, after a small allowance for SU(3) symmetry breaking²⁷, yields $\sin\theta_c = 0.238 \pm 0.005$. Another approach to θ_c comes from K_{e3} -decay which, interpreted conventionally using the Klein-Gordon equation for the pion, yields²⁸ $\sin\theta_c = 0.223 \pm 0.003$. Since K-decay is not so closely connected to the nucleon-decay problem as is hyperon decay, since it has fewer internal consistency checks and since there is some doubt about the appropriate treatment of the pion²⁹, we do not try to combine the two values of $\sin\theta_c$ but rather accept the K_{e3} value as evidence that the hyperon value may be a bit on the high side and so reduce the latter by its own standard deviation and adopt

$$\sin\theta_c = 0.233 \pm 0.005$$

Muon decay

The mean-life of the muon, τ_μ , is given by

$$\tau_\mu = \frac{192\pi^3 \hbar^7 (1 - \delta_\mu^a)}{m_\mu^5 c^4 (1 - 8m_e^2/m_\mu^2) g_\mu^R{}^2}$$

where δ_μ^a is the "outer" radiative correction of order α , the analogue of δ_β^a , and is given by^{29,30}

$$\delta_\mu^a = -(\alpha/2\pi) (\pi^2 - (25/4))$$

δ_μ^a , and higher corrections have been evaluated only incompletely³¹ but seem unlikely to introduce changes of more than 1 part in 10^4 in τ_μ . g_μ^R contains the effect on τ_μ of the anatomy of the β -decay process

$$g_\mu^R = g_\mu (1 + \frac{1}{2} \Delta_\mu^a)$$

Recent experiments give

$$\begin{aligned} \tau_\mu &= 2197.3 \pm 0.3 \text{ ns} \quad (\text{ref. 32}) \\ &= 2197.11 \pm 0.08 \text{ ns} \quad (\text{ref. 33}) \end{aligned}$$

so that

$$g_\mu^R = (1.4358 \pm 0.0001) \times 10^{-49} \text{ erg cm}^3$$

where the error has been inflated to take account of the uncertainties in δ_μ^a .

$$\Delta_{\beta\gamma}^a - \Delta_\mu^a$$

We bring together the various values quoted above to find

$$\Delta_{\beta\gamma}^a - \Delta_\mu^a = 2 \left\{ \frac{g_{\beta\gamma}^R}{g_\mu^R \cos\theta_c} - 1 \right\} = (2.19 \pm 0.27) \%$$

which we could confront, to find \bar{Q} , with Sirlin's above-quoted theoretical expression for $\Delta_{\beta\gamma}^a - \Delta_\mu^a$, if we knew m_Z .

M_Z

Within the Salam-Weinberg scheme, that we have adopted, the mass, m_Z , of the neutral intermediate vector boson that figures in Sirlin's formula for $\Delta_{\beta\gamma}^a - \Delta_\mu^a$ is given by: $m_Z = 74.6/\sin(2\theta_w)$ GeV; θ_w is the Weinberg angle that relates the weak and electromagnetic coupling constants. The scheme is encouraged by the "neutral current" neutrino interactions that for muon-less hadron-producing events, within a quark-parton model such as we consider here, give $\sin^2\theta_w = 0.39 \pm 0.05$ (ref. 34). $\bar{\nu}_e e$ -scattering (to which charged currents can also contribute) gives $\sin^2\theta_w < 0.3$ (ref. 35). $\bar{\nu}_\mu e$ -scattering is not

inconsistent with $\sin^2\theta_w \simeq 0.25$ (ref. 34 and D. H. Perkins, private communication), somewhat smaller and somewhat larger values being admissible. For our present purpose we therefore use:

$$74.6 < m_Z < 100 \text{ GeV}$$

where 100 GeV corresponds to $\sin^2\theta_w \simeq 0.17$ which appears to be safely on the low side.

\bar{Q}

Our experimental $\Delta_{\beta\gamma}^a - \Delta_\mu^a$ and the above-adopted m_Z are confronted with Sirlin's formula in Fig. 1; we derive: $\bar{Q} = 0.20 \pm 0.10$. (The largest component in the error comes from that in θ_c .)

We repeat the various cautionary remarks about our reliance on the hypotheses listed above, in particular on the Salam-Weinberg scheme, and on Sirlin's derivation of his formula, but within these limits our value of \bar{Q} can be treated as a definite parameter.

Quark charges

$\bar{Q} = 0.20 \pm 0.10$ is consistent with $\bar{Q} = 1/6$ expected for the classical quark model; it is inconsistent with $\bar{Q} = 1/2$ as for integral quarks. Other data relevant to "quark charges" come from a comparison of deep inelastic neutrino and electron scattering; the two show good internal consistency within the quark-parton model and their comparison then permits the statement, if there are no strange constituents to the nucleon, which is a good assumption in view of the evident lack of anti-matter remarked on above: $\bar{Q}^2 = 0.28 \pm 0.03$ (ref. 6) which is consistent with the expectation of the classical quark model: $\bar{Q}^2 = 5/18$.

Putting together \bar{Q} and \bar{Q}^2 , the "experimental" values of the separate quark charges become

$$\begin{aligned} Q_p &= 0.69 \pm 0.07 \\ Q_n &= -0.29 \pm 0.15 \end{aligned}$$

to be compared with the classical values: $Q_p = 2/3$, $Q_n = -1/3$.

It is the especial virtue of the present approach that it is sensitive to \bar{Q} rather than to \bar{Q}^2 .

Experimental data

J. M. Freeman, W. E. Burcham and their colleagues at AERE, Harwell have, for many years, carried out accurate measurements on super-allowed Fermi β -decay. More recently, important contributions have also come from D. E. Alburger, J. C. Hardy and their colleagues at Brookhaven National Laboratory and Chalk River. Old inconsistencies have now disappeared; the present situation, summarised in Table 1, where t has been corrected for branching and electron capture, is one in which considerable confidence may be reposed.

E_0 is the maximum kinetic energy of the positron. f is defined as $f^R(1-\epsilon)$. The ϵ -values have been taken from Table 2 and an error of $\pm 0.1\%$ associated with each. $f t$ should then be a constant if the vector current is conserved; its mean value

Table 2

A	$\epsilon_1\%$ (ref. 43)	$\epsilon_1\%$ (ref. 44)*	$\epsilon_1\%$ (ref. 24)	$\epsilon_2\%$ (ref. 24)	$\epsilon\%$
14	0.04	0.04	0.15	0.05	0.13
26	0.10	0.11	0.12	0.07	0.18
34	0.17	0.18	0.19	0.23	0.41
38	0.21	0.21	0.21	0.16	0.37
42	0.25	0.25	0.31	0.13	0.40
46	0.30	0.29	0.17	0.04	0.29
50	0.35	0.33	0.21	0.03	0.33
54	0.40	0.38	0.27	0.04	0.39

* And A. M. Lane, private communication.

is $\bar{f}t = 3,087.7 \pm 2.3$ s with $\chi^2/\nu = 0.56$ so internal consistency is good.

Alternatively, we may follow the suggestion of the Ademollo-Gatto theorem¹⁹ namely that ϵ should go, *ceteris paribus*, as the square of the mass-splittings within the isomultiplets which, over the range of Table 1, means roughly as $Z^{1.75}$. Fitting to the form: $f^R t = (f^R t)_{Z=0} + aZ^{1.75}$, gives $(f^R t)_{Z=0} = 3,088.5 \pm 3.8$ s in good agreement with $\bar{f}t$. We adopt $3,087.9 \pm 3.5$ s; this leads to the $g_{\beta V}^R$ -value quoted in the text.

The ϵ -values

There is a long history to computation of the ϵ -values⁴¹. Recent estimates are shown in Table 2. ϵ splits into two parts: ϵ_1 is due to the one-body Coulomb force and ϵ_2 to the two-body Coulomb force. The three estimates of ϵ_1 , made by very different methods, agree well among themselves (the third of them has been halved to allow more properly for the parentage spectrum⁴³, except for ⁴²Sc where the parentage is unique). It seems that $\epsilon = \epsilon_1 + \epsilon_2$ as quoted should be reliable to $\pm 0.1\%$.

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In vitro synthesis of tRNA precursors and their conversion to mature size tRNA

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Two *Escherichia coli* tRNA gene clusters, $tRNA_1^{Tyr}$ (su_3^+ and su_3^-) and $tRNA_2^{Tyr}$, $tRNA_2^{Gly}$ (su_3^+), $tRNA_3^{Thr}$, were transcribed in a purified *in vitro* system. Evidence indicates that the adjacent tRNA genes are transcribed together as a common precursor of large size, which, on incubation with crude cell extracts, yields mature tRNA molecules.

It is now generally established that tRNA genes of bacterial and mammalian cells as well as those of bacteriophage T4 are transcribed into large RNA precursor molecules which are subsequently cleaved by specific nucleases to yield mature size tRNA¹⁻⁶. Because of the transient nature of the tRNA precursors, however, only in a few cases have such molecules been isolated *in vivo*^{1,7} and some of them may represent already partially cleaved precursors^{8,9}. Transcription of tRNA genes *in vitro* by purified RNA polymerase has the advantage of producing completely unmodified tRNA precursors and provides an opportunity to study in detail the processing and modification of primary transcription products.

The structural genes for some tRNA molecules are often present on the *E. coli* chromosome as clusters containing identical genes^{10,11}, or genes specifying different tRNAs¹². Two such tRNA gene clusters are being studied: the duplicate genes coding for $tRNA_1^{Tyr}$ (su_3^+ and su_3^-) carried by transducing phage $\phi 80psu_3^{+,-}$ (ref. 13) and the $tRNA_2^{Gly}$ (su_3^+) $tRNA_3^{Thr}$, $tRNA_2^{Tyr}$ genes carried by $\lambda h80T$ phage DNA¹². *In vivo*^{8,14} and *in vitro*^{15,16} studies suggest that these tRNA genes may be transcribed as large RNA precursors. In the

present report the multiple tRNA genes carried by $\phi 80psu_3^{+,-}$ and $\lambda h80T$ phage DNA were transcribed *in vitro* by *E. coli* RNA polymerase as polycistronic tRNA precursors. These precursors were cleaved on incubation with a supernatant fraction prepared from *E. coli*, yielding mature size tRNA molecules. The process of precursor cleavage was analysed using polyacrylamide gel electrophoresis. It was found that the formation of tRNA takes place by way of intermediate size RNA molecules. The mature size tRNA and some of the intermediate precursors were found, using fingerprint techniques, to be similar or identical to those made *in vivo*.

Transcription and processing of $tRNA_1^{Tyr}$ precursor

$\phi 80psu_3^{+,-}$ DNA carrying both suppressor and wild-type $tRNA_1^{Tyr}$ genes was transcribed *in vitro* by RNA polymerase in a reaction mixture containing α -³²P-GTP. After 30 min incubation at 37 °C, RNA synthesis was terminated by the addition of actinomycin D and DNase I. The formation of mature size $tRNA_1^{Tyr}$ from larger precursors was studied by following the processing of the *in vitro* $\phi 80psu_3^+$ RNA transcripts by crude *E. coli* S100 extracts as a function of incubation time.

The synthesised α -³²P-GMP-labelled RNA was extracted from a portion of the reaction mixture (zero time of processing). The rest of the reaction mixture, was incubated with S100 extract from *E. coli*. At different time intervals (5, 10, 20 and 40 min) equal aliquots were removed, the RNA extracted by phenol and precipitated by ethanol. The RNA preparations were separated by electrophoresis on a 5% polyacrylamide-7 M

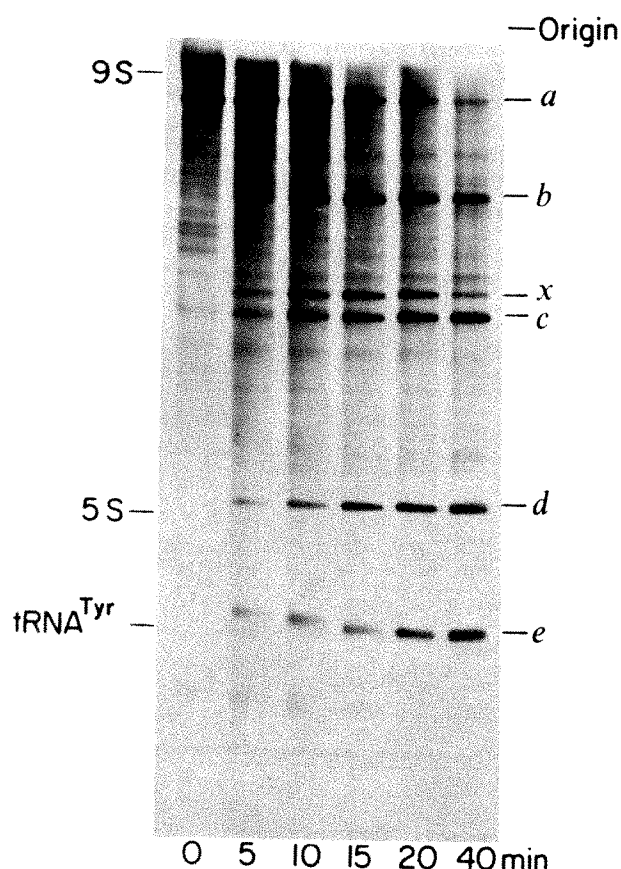


Fig. 1 Autoradiogram of acrylamide gel separations of cleavage products of RNA transcribed *in vitro* on $\phi 80psu_3^{+/-}$ DNA. $\phi 80psu_3^{+/-}$ DNA (20 μ g) was transcribed in 200 μ l reaction mixture containing: 0.05 M Tris-HCl (pH 7.9), 0.01 M $MgCl_2$, 0.075 M KCl, 1 mM dithiothreitol, 0.4 mM each of ATP, CTP and UTP, 0.3 mM $\alpha\text{-}^{32}P\text{-GTP}$ (2.3 Ci mmol $^{-1}$; Amersham, England) 12 μ g ρ termination factor and 60 U RNA polymerase (1,600 U mg $^{-1}$). The synthesis was initiated after 5 min pre-incubation at 38 $^{\circ}C$ by addition of nucleoside triphosphates. After 30 min incubation at 38 $^{\circ}C$ the reaction was stopped by the addition of 20 μ g actinomycin D and 4 μ g DNase I (Worthington, Freehold, New Jersey). RNA was extracted from a 30 μ l aliquot of the reaction mixture and run on acrylamide gel to give the electrophoretic pattern for zero time of processing. The rest of the mixture was incubated at 37 $^{\circ}C$ with 400 μ g S100 extract. Aliquots were removed at the times indicated in the figure and fractionated by acrylamide gel electrophoresis on 40 \times 20 cm 5% gels (20:1 acrylamide-bis-acrylamide) containing 7 M urea, in Tris-borate buffer¹⁷ at 175 V for 16–19 h. 9S globin mRNA, 5S RNA and tRNA^{Tyr} were run separately as reference markers and were located by staining the gel with Stains all (Eastman-Kodak). DNA-dependent RNA polymerase was prepared from *E. coli* MRE600 cells¹⁸ and further purified by low salt glycerol gradient centrifugation¹⁹. Termination factor ρ was prepared from *E. coli* MRE600 cells as described by Roberts²⁰. S100 supernatant fraction was prepared from log-phase *E. coli* MRE600 cells suspended in buffer (0.01 M Tris-acetate, pH 7.5, 0.014 M $MgCl_2$, 0.06 M KCl, 6 mM 2-mercaptoethanol) and disrupted in a French press. The extract was centrifuged for 90 min at 105,000g in a Spinco ultracentrifuge and the S100 supernatant obtained was dialysed against the same buffer and stored at $-20^{\circ}C$.

urea gel. An autoradiogram of the gel separation for the RNA preparations is presented in Fig. 1. A single RNA band is observed in the non-processed $\phi 80psu_3^{+/-}$ RNA (band *a* at 0 time of incubation). On incubation with the S100 extract, the intensity of this band decreases. Band *e*, with the mobility of tRNA^{Tyr}, appears only after addition of the S100 extract and increases in intensity with the time of incubation. Attention should be drawn to the fact that band *e* RNA reaches the size of mature tRNA^{Tyr} only after 15–20 min incubation. The varied

dispersity and larger size of band *e* RNA at shorter periods of incubation may be due to insufficient trimming of the additional nucleotide sequences by an exonuclease activity present in the S100 extract. Figure 1 also shows that the maturation of tRNA^{Tyr} is a stepwise process involving the formation of intermediate size RNA molecules. Concomitant with the disappearance of band *a* during incubation with the S100 extract, additional RNA bands *b*, *c*, *d* and *x* are formed, the intermediate character of which is suggested by the fact that during incubation their intensity increases, reaches a maximum and decreases again. This behaviour is clearly seen in the case of band *x* for the incubation periods covered by Fig. 1. For sufficiently long incubation periods all intermediate RNA bands virtually disappear (data not shown).

Transcription and processing of tRNA^{Tyr}, tRNA^{Gly}, tRNA^{Thr} precursor

λ h80T DNA was transcribed *in vitro* by RNA polymerase in a reaction mixture containing $\alpha\text{-}^{32}P\text{-GTP}$. The formation of tRNA molecules from precursors was studied by following the cleavage of the *in vitro* λ h80T RNA by *E. coli* S100 extracts as a function of incubation time. Figure 2 shows that the primary transcription product of λ h80T DNA (0 time of incubation with S100 extract) is composed of RNA molecules of varied size, which remain at the origin or migrate in the 9S region of the gel, and of three distinct bands *a*, *c* and *g*. On incubation with S100 extract, two 4S RNA bands (*e* and *f*) appear, their intensity increasing with time. Band *e* has a mobility corresponding to a tRNA^{Tyr} molecule. Band *f*, as shown later, is actually composed of two tRNA species, tRNA^{Thr} and tRNA^{Gly}, which do not separate on the urea-containing polyacrylamide gel. As observed previously for the formation of tRNA^{Tyr} from the $\phi 80psu_3^{+/-}$ DNA transcript (Fig. 1), the tRNA species in bands *e* and *f* (Fig. 2) appear first as diffuse bands and reach the mature tRNA size only after 15 min incubation. The

Fig. 2 Autoradiogram of acrylamide gel separation of cleavage products of RNA transcribed *in vitro* on λ h80T DNA. RNA synthesis and cleavage by S100 extract was as described in Fig. 1.

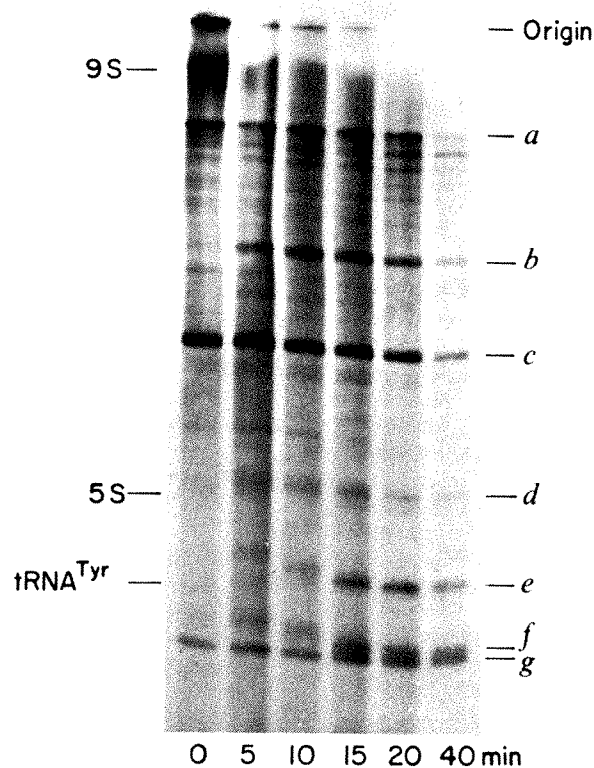


Table 1 Nearest-neighbour analysis of T_1 RNase oligonucleotides from *in vitro* tRNA $_1^{Tyr}$ and tRNA $_2^{Tyr}$

Spot no.	Corresponding tRNA $_1^{Tyr}$ and tRNA $_2^{Tyr}$ sequence	Nearest-neighbour predicted	Nearest-neighbour found
1	G!	G	G
2	G	G	G
3	CG(G)	C,G	C,G
4	CCG	C	C
5	AG	A	A
6	CAG	A	A
7	AAG(G)	A,G	A,G
8	CCAAG(G)	A,G	A,G
9	UG(G)	U,G	U,G
10	pGp	G	G
11	UG!(G)	U,G	U,G
12	pGp!	G	G
13	—	—	U
14	TψCG	C	C
15	UUCCCG	C	C
16	UCAUCG*	C	C
17	ACUUCG	C	C
18	UAAAψCUG	U	U
19	ACUCUAAAψCUG*	U	U
20	ACUG	U	U
21	UCACAG†	A	A

Oligonucleotides were eluted from the two-dimensional fingerprints and hydrolysed with 0.3 M KOH at 37 °C. Resulting nucleotides were separated by paper electrophoresis at pH 3.5 and identified by autoradiography.

* Fragment derived from tRNA $_1^{Tyr}$ (ref. 22).

† Fragment derived from tRNA $_2^{Tyr}$ (ref. 22).

appearance of the tRNA bands is accompanied by the cleavage of band *a* (Fig. 2). For longer periods of incubation the disappearance of the other bands was also observed (data not shown).

Sequence analysis of tRNA molecules synthesised *in vitro*

The synthesised tRNA molecules were identified by the two-dimensional fingerprint of T_1 RNase digestion fragments²¹. As *in vitro* transcription was carried out with α -³²P-GTP and T_1 RNase cleaves the RNA adjacent to GMP residues, each fragment obtained contains a labelled 3'-terminal GMP. The two-dimensional fingerprint of the tRNA synthesised *in vitro* should therefore be the same as that obtained with the respective uniformly labelled *in vivo* tRNA species. The only exception is the 3'-terminal fragment of the tRNA which contains no GMP and will not be labelled in the *in vitro* transcript. Autoradiograms of the two-dimensional T_1 RNase fingerprints of band *e* (tRNA $_1^{Tyr}$) from Fig. 1 and band *e* (tRNA $_2^{Tyr}$) from Fig. 2 are presented in Fig. 3*a* and *b*, respectively. The two fingerprints are similar to each other and to the T_1 RNase fingerprint of *in vivo* *E. coli* tRNA $_1^{Tyr}$ described by Goodman *et al.*²². The oligonucleotides isolated in the fingerprints were extracted from the DEAE paper and the nearest-neighbour distribution of the labelled GMP in the fragments was determined by alkaline hydrolysis. The results for both tRNA $_1^{Tyr}$ and tRNA $_2^{Tyr}$ synthesised *in vitro* are presented in Table 1 and show that given its position on the fingerprint, each oligonucleotide yielded the nearest-neighbour nucleotide expected, assuming it to be derived from tRNA Tyr (ref. 22).

The tRNA $_2^{Tyr}$ transcribed on λ h80T DNA differs from the tRNA $_1^{Tyr}$ species transcribed on ϕ 80 $psu_3^{+/-}$ DNA by the presence of fragment UCACAG (Fig. 3*b*, spot 21). The tRNA $_2^{Tyr}$ fingerprint also contains the fragments ACUG (spot 20) and UAAAψCUG (spot 18), both derived from the anticodon sequence ACUGUAAAψCUG. Because of replacement of G by a C in this sequence in tRNA $_1^{Tyr}$ su_3^+ the fragment is not cleaved by T_1 RNase and appears as oligonucleotide 19 in Fig. 3*a*. As ϕ 80 $psu_3^{+/-}$ DNA codes both for su_3^- and su_3^+ species of tRNA $_1^{Tyr}$, however, the fingerprint in Fig. 3*a* also contains the spots ACUG (20) and UAAAψCUG

(18) derived from su_3^- . The T_1 RNase-resistant fragment C 2'-O-methyl GG, normally found in *in vivo* tRNA Tyr , is absent from both tRNA $_1^{Tyr}$ and tRNA $_2^{Tyr}$ synthesised *in vitro* (Fig. 3*a* and *b*); the CG (spot 3) observed in both fingerprints derives from the unmodified sequence CCG.

To determine the 3'-terminal sequences of tRNA $_1^{Tyr}$ we have synthesised ϕ 80 $psu_3^{+/-}$ RNA using α -³²P-CTP. T_1 RNase fingerprints of α -³²P-CMP-labelled band *e* RNA reveal a spot with a mobility identical to that of the oligonucleotide AAUCCUCCCCCACCACCAOH observed for *in vivo* tRNA Tyr (ref. 22). The nearest-neighbour distribution of the labelled CMP in this fragment was as expected 2A:2U:7C. Band *d* RNA transcribed on the ϕ 80 $psu_3^{+/-}$ DNA template which migrates in the 5S region of the acrylamide gel (Fig. 1)

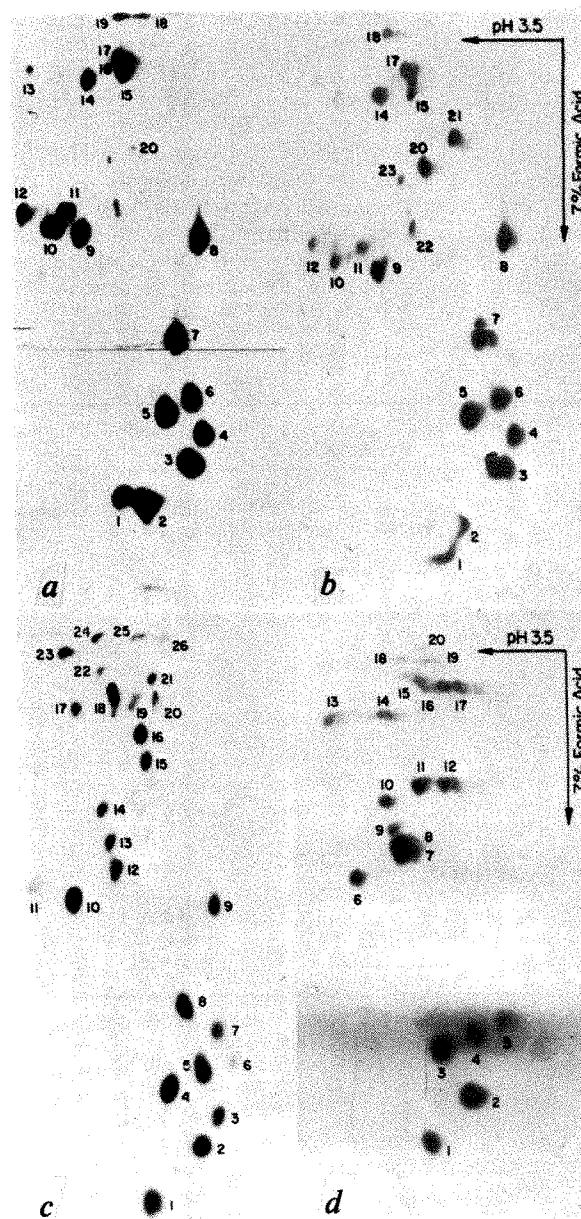


Fig. 3 Autoradiogram of fingerprints of tRNA molecules synthesised *in vitro*. *a*, tRNA $_1^{Tyr}$ (su_3^+ and su_3^-) transcribed on ϕ 80 $psu_3^{+/-}$ DNA (band *e* from Fig. 1). *b*, tRNA $_2^{Tyr}$ transcribed on λ h80T DNA (band *e* from Fig. 2). *c*, tRNA $_1^{Tyr}$ (5S) precursor transcribed on ϕ 80 $psu_3^{+/-}$ DNA (band *d* from Fig. 1). *d*, Mixed fingerprint of tRNA Thr and tRNA Gly transcribed on λ h80T DNA (band *f* from Fig. 2). Fingerprint analysis was carried out by extraction of the RNA bands from the gel by homogenisation in 0.5 M NaCl, 0.1 M Tris-HCl, pH 8.0, and 0.01 M EDTA, digestion by T_1 RNase and two-dimensional electrophoresis according to Sanger *et al.*²¹.

was also analysed by the fingerprint method. The autoradiogram presented in Fig. 3c identifies this RNA as a $\text{tRNA}_{1^{\text{Tyr}}\text{su}_3^+}$ precursor. The nearest-neighbour analysis of the T_1 RNase oligonucleotides obtained from the fingerprint separation is similar to that of the *in vivo* 5S precursor of $\text{tRNA}_{1^{\text{Tyr}}\text{su}_3^+}$ described by Altman and Smith⁷. The fingerprint of the 5S $\text{tRNA}_{1^{\text{Tyr}}}$ precursor synthesised *in vitro* does not contain the ACUG fragment and seems therefore to be a precursor of $\text{tRNA}_{1^{\text{Tyr}}\text{su}_3^+}$ only. It contains all the fragments observed in Altman's *in vivo* $\text{tRNA}_{1^{\text{Tyr}}\text{su}_3^+}$ precursor except the nucleotide pppGp representing the 5' terminus of the molecule. The fingerprint of the *in vitro* product contains in addition small amounts of pGp (spot 11) and a number of unidentified fragments (spots 6, 11–15, 22, 23, 25, 26) which were not observed in the *in vivo* $\text{tRNA}_{1^{\text{Tyr}}}$ precursor (Fig. 3c).

Transcription of λh80T DNA followed by S100 cleavage of the RNA (Fig. 2) was shown to produce in addition to band *e*, identified as $\text{tRNA}_{2^{\text{Tyr}}}$, band *f*, which is expected to contain the two tRNA species, tRNA_{Gly} and tRNA_{Thr} . Figure 2 shows that band *g*, present in the precursor even before the processing, behaves like a stable RNA species and migrates on the gel very close to band *f*. It was necessary therefore to repurify band *f* before the fingerprint analysis. The cut-out gel of band *f* was transplanted on top of a 16% acrylamide gel containing 7 M urea and was separated by electrophoresis from the faster running traces of band *g*. The fingerprint of band *f* (Fig. 3d), seems to contain the mixed sequences of both tRNA_{Gly} and tRNA_{Thr} .

Table 2 Nearest-neighbour analysis of T_1 RNase oligonucleotides from *in vitro* tRNA_{Gly} and tRNA_{Thr}

Spot no.	Corresponding tRNA_{Gly} and tRNA_{Tyr} sequence	Nearest-neighbour predicted	Nearest-neighbour found
1	G	G	G
2	CG(G)	C,G	C,G
3	AG(G)*	A,G	A,G
4	CAG*	A	A
5	CCCG†	C	C
6	UG*	U	U
7	UCG(G)*	C,G	C,G
8	CUG	U	U
9	DAG*	A	A
10	AUG†	U	U
11	UAAG(G)*	A,G	A,G
12	CAUCG†, CUCAG*	C,A	C,A
13	DDG(G)*	U,G	U,G
14	TψCG	C	C
15	AUAUAG*	A	A
16	AUUCGG*, AAUCUG*	C,U	C,U
17	CCUAUCAG*, CACCCUUG(G)†	A,G,U	A,G,U
18	UAUAAUG(G)†	U,G	U,G
19	CUAUUACCUCAG†	A	A
20	CCUUCUAG†	A	A

* Fragment derived from tRNA_{Thr} (ref. 24).

† Fragment derived from tRNA_{Gly} (ref. 23).

In Table 2, the nearest-neighbour analysis of the different fragments from the fingerprint is compared with that expected for the respective *E. coli* tRNA species^{23,24}. The T_1 oligonucleotide $7^{\text{m}}\text{GUCCG}^{24}$ is absent from the fingerprint of the tRNA_{Thr} synthesised *in vitro*. Due to the presence of a non-modified G at the 5'-terminus, this fragment is degraded by T_1 to form UCG which appears as spot 7. Note that the pGp fragment, normally derived from the 5'-terminus of the tRNA_{Thr} and tRNA_{Gly} (refs 23 and 24) is absent from the fingerprint of the *in vitro* tRNAs synthesised *in vitro*. A tentative explanation for the absence of pGp at the 5'-terminus of *in vitro* tRNA_{Thr} and tRNA_{Gly} may be a wrong precursor cleavage by RNase P.

The RNA molecules migrating as band *g* (Fig. 2) were also

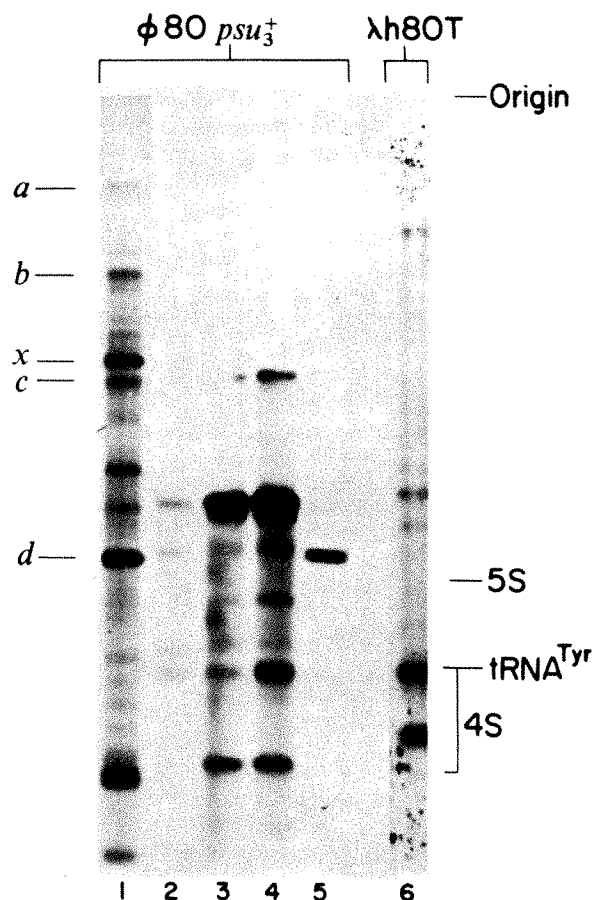


Fig. 4 Autoradiogram showing formation of tRNA from isolated precursor RNA. RNA bands were extracted from the gel separation of $\phi 80\text{psu}_3^{+/-}$ RNA or λh80T RNA synthesised *in vitro*, incubated for 60 min with S100 extract (10 min for band *a* from Fig. 1) and fractionated by polyacrylamide gel electrophoresis: 1, 2, 3, 4 and 5 refer to bands *a*, *b*, *x*, *c* and *d* from Fig. 1; 6 refers to band *a* from Fig. 2.

studied by the fingerprint method but did not seem to contain tRNA sequences.

Polycistronic tRNA precursors

Figure 1 suggests that RNA band *a* is a primary transcription product of the $\text{tRNA}_{1^{\text{Tyr}}}$ genes on $\phi 80\text{psu}_3^{+/-}$ DNA and that bands *b*, *x*, *c* and *d* are intermediate precursors of $\text{tRNA}_{1^{\text{Tyr}}}$. To provide further support for this assumption we have extracted the RNA bands from the gel and subjected them to cleavage by incubation with S100 extract. Figure 4 shows that a short incubation (10 min) of band *a* with S100 extract generates the intermediate RNA bands *b*, *x*, *c* and *d* as well as some $\text{tRNA}_{1^{\text{Tyr}}}$. RNA bands *b*, *x* and *c* all produce $\text{tRNA}_{1^{\text{Tyr}}}$ band on processing. Although band *d* RNA has been shown by fingerprint analysis (Fig. 3c) to carry $\text{tRNA}_{1^{\text{Tyr}}}$ sequences it was not cleaved after extraction from the gel and incubation with the S100 extract (Fig. 4). This behaviour is unexpected in view of the fact that this band does not seem to accumulate significantly when processing by S100 extract is carried out directly in the transcription mixture (legend to Fig. 1). The possible reasons for this observation are being investigated.

The isolation and cleavage of band *a* RNA from λh80T DNA transcript (Fig. 2) generates two RNA bands in the 4S region of the gel, one corresponding to $\text{tRNA}_{1^{\text{Tyr}}}$ and the other to the mixture tRNA_{Gly} , tRNA_{Thr} (Fig. 4, gel 6). It may therefore be concluded that the three tRNA species coded by λh80T DNA are present on a common polycistronic precursor. Polycistronic precursors for the tRNA gene clusters carried

by $\phi 80psu_3^{+/-}$ DNA and $\lambda h80T$ DNA have not yet been shown *in vivo*. The dicistronic RNA molecule containing tRNA^{Gly}, tRNA^{Thr} isolated by Carbon⁸ may be only a part of a primary precursor. The transcription *in vitro* of the adjacent genes of tRNA^{Trp}, tRNA^{Gly}, tRNA^{Thr} and tRNA^{Trp} (su_3^+ and su_3^-) as polycistronic precursors provides direct evidence that these genes belong to a single transcriptional unit.

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Is the evolution of insulin Darwinian or due to selectively neutral mutation?

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A model for the evolution of insulin mainly in terms of adaptive processes is discussed. The model depends critically on the relationship of sequence changes to the three-dimensional structure and the role of various parts of this structure in the conversion of the proinsulin molecule to the active form, the storage of insulin, its transport to the site of action and its interaction with a receptor.

SEQUENCES of functionally equivalent proteins provide the best evidence for selectively neutral mutations. As the knowledge of proteins increases, however, more detailed attempts to discern the relative importance of adaptive and neutral mutations in evolution may become possible. In particular, the detailed study of the role of different amino acid residues in a class of proteins and the effects of sequence changes on their physical and physiological properties is an essential prerequisite to an assessment of arguments for and against neutral mutations^{1,2}. Three principal arguments are given in support of neutral mutations. Kimura³ suggested that the substitutional load due to the high rate of evolution would have been intolerable unless most of the mutations had been neutral in natural selection. The main argument of King and Jukes⁴ is based on the observation that the rates of evolution of individual proteins are uniform and the idea that most amino acid differences between species are functionally insignificant. This uniform rate and the apparent latitude for random genetic change at the molecular level is said to hold for cytochrome *c*, haemoglobin, histone and also for insulin with the exception of guinea pig and coypu insulins. Markussen⁵ has calculated evolutionary rates for insulins based on nucleotide differences and considered insulin evolution in terms of predominantly neutral mutation.

We feel that the above views result from an oversimplification of the meaning of 'functional insignificance' and depend on the use of average rates of change without sufficient regard to the location and chemical nature of

sequence changes. We wish to discuss a model for the evolution of insulin mainly in terms of adaptive processes. We do not exclude the possibility of some neutral change; rather we wish to provide an alternative explanation for the evolution of insulin and to caution conclusions made on the basis of uniform rates and changes that seem not to not affect function.

Insulin is a protein hormone produced and stored in the B cells of the islets of Langerhans of the pancreas. It is released into the circulation from membrane-limited intracellular storage granules in response to raised blood glucose levels and facilitates removal of glucose and other metabolites to the tissues. Insulin is synthesised as a single chain precursor—proinsulin. Within the storage granules, enzyme action removes a peptide about 30 residues long—the connecting peptide—leaving the active hormone. Most insulins comprise 51 amino acids, 21 in the A chain and 30 in the B chain. The chains are linked by two disulphide bonds. Insulin binds to specific receptor sites on the target cell surface but the details of how a biological response is initiated are not fully understood.

The amino acid sequences of 28 insulins (from the jawless hagfish through bony fishes such as cod and birds such as the turkey, to mammals) have been determined (Table 1) and these insulins are active in other test animals. The rate of amino acid substitution for most insulins has been in the order of 1×10^{-8} per locus per year (Paulings).

The three-dimensional structure of pork insulin has been described in detail by Hodgkin *et al.*^{7–9}. The biosynthesis of proinsulin¹⁰, the storage in the B cell granules^{11,12} and the receptor binding^{13–15} have been studied widely. Our views depend critically on the relation of sequence changes to the three-dimensional structure and the role of various parts of this structure in the different aspects of the physiology of insulin. In fact several characteristics of insulin may be of importance in determining fitness. These might include the correct folding of the newly synthesised proinsulin molecule and its conversion to an active form, the storage of insulin, its transport to the site of action and its interaction with a receptor. Most of these characteristics

will depend on the integrity of the three-dimensional structure, possibly on the quaternary structure and certainly on the solubility of insulin, its interaction with other proteins in the organism, its thermodynamic stability and its resistance to enzymatic degradation. All these aspects could act as restraints at various stages of evolution and changes in the anatomy and physiology of different animals may change these restraints. For instance, the observation¹⁶ that the location of the insulin-producing B cell has moved from the gut wall in tunicates to clusters of cells at the anterior end of the gut in Agnatha and further to the specialised organ, the pancreas, enclosing both endocrine and exocrine (especially proteolytic) functions in higher vertebrates may give us some clue to the changes that could have occurred in storage restraints on the insulin molecule.

For insulin we can now begin to define the above characteristics in terms of the tertiary structure and the amino acid sequences. In this discussion we include the unpublished results of X-ray and circular dichroism studies together with the biological properties of a number of different insulins of known sequence.

Structure and function

X-ray analysis shows that the pig insulin monomer has a well defined, globular three-dimensional structure with an hydrophobic core and two predominantly hydrophobic surfaces (Fig. 1). These surfaces are important for association of the insulin molecules first as a dimer and then in the presence of zinc to a hexamer. The surface of the hexamer contains most of the hydrophilic residues. The hexamer has the shape of a torus with two zinc atoms bound in the central, cylindrical channel (Fig. 2). The amino acids responsible for receptor binding and stimulation of a biological

response are located by studies of chemically modified and semi-synthetic insulins. The monomer is the active form; although receptor binding involves some of the hexamer surface it probably requires certain of the hydrophobic residues involved in dimerisation also (Fig. 1). Maintenance of the tertiary structure seems to be essential for receptor binding.

The connecting peptide of proinsulin links residues A1 and B30 (Fig. 1) which are only 10 Å apart although the peptide is about thirty amino acids long. The proinsulin molecules hexamerise¹⁷, thus the connecting peptide must lie on the hexamer surface. The role of the connecting peptide seems to be not only concerned with the folding of proinsulin to the correct tertiary structure but also to increase the solubility of the hexamer in the presence of high zinc concentrations¹⁸. When the connecting peptide is cleaved the insulin crystallises as granules; with the exception of some hystricomorph insulins and probably hagfish insulin, the granules contain zinc insulin hexamers. The role of the zinc insulin hexamers seems to be to provide a storage form which is thermodynamically stable and more resistant to enzymatic degradation than the unassociated form. The zinc-containing granules of different species are usually crystalline in appearance but electron microscopy shows that they have a varied morphology, symmetry and molecular packing^{11,12}. This variation in granule structure finds a parallel in the variation of crystal shapes and forms in the laboratory. It seems that the ease of crystallisation of zinc insulin hexamers may be a reflection of the granular structure found in the B cell, and the insulin that can form zinc insulin hexamers may have evolved as a stable storage form. The hexamers are probably unrelated to receptor binding. Thus it can be seen that there are many facets of

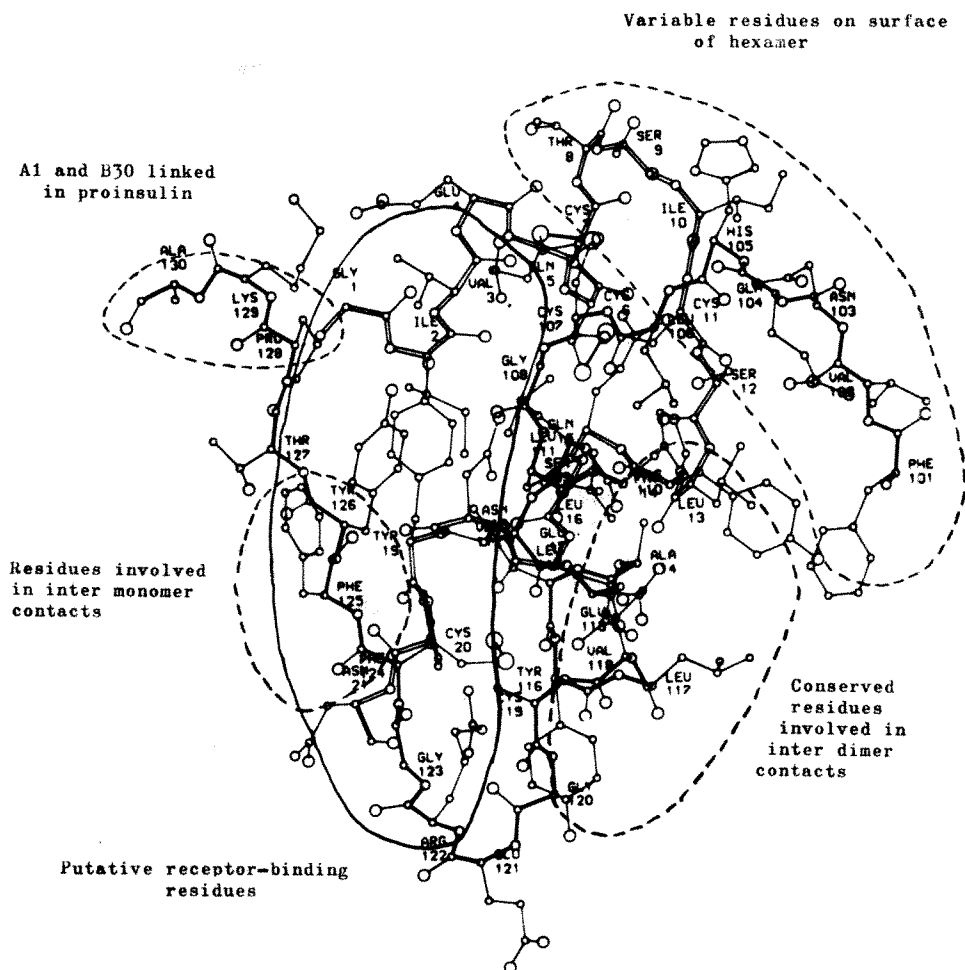


Fig. 1 View of the insulin monomer indicating how the surface may be described in terms of various areas involved in activation from the prohormone, binding to the receptor and self association.

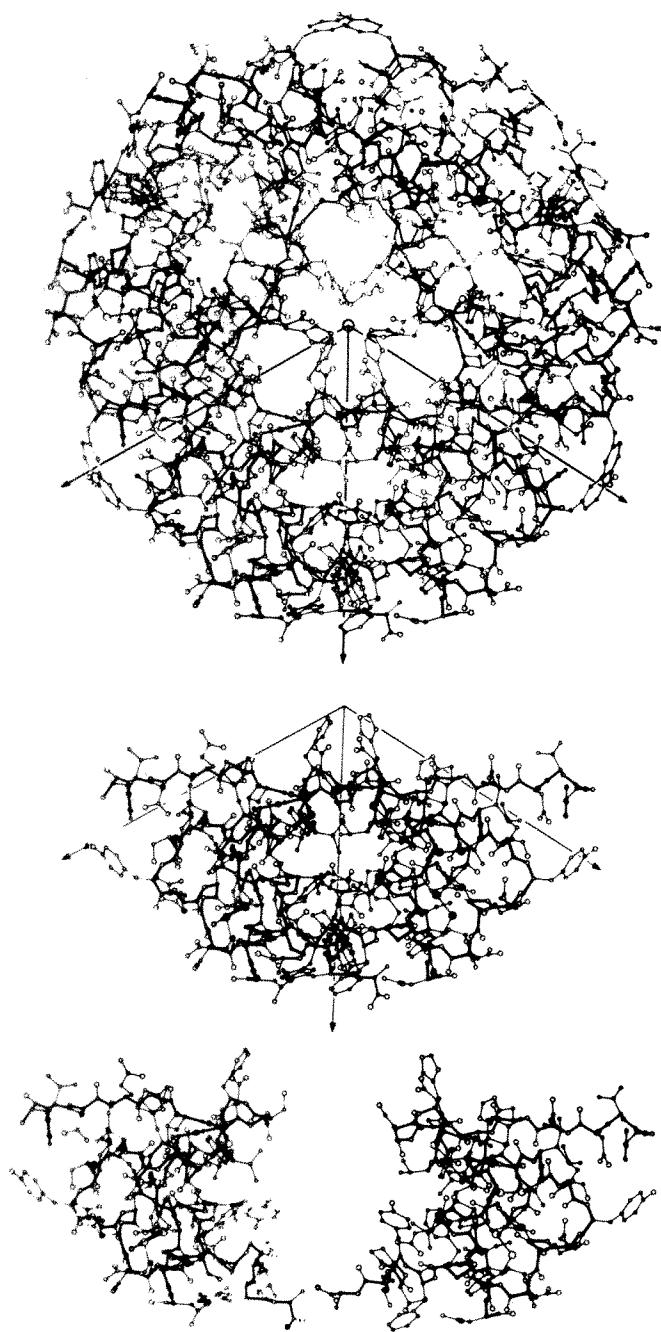


Fig. 2 Formation of the zinc insulin hexamer.

adaptation in the insulin molecule, only one of which is receptor binding.

Sequences and structure

The sequences available indicate that all insulins might attain the tertiary structure found for pig insulin. The disulphide bridges and the hydrophobic core of the monomer are invariant. The residues which vary do not have the same location in different classes of animals. In the mammals, with the exception of the hystricomorphs, all insulins can form dimers and zinc insulin hexamers. The most highly variable residues are A8–A10 and B29 and B30. These residues are close together on the hexamer surface; they are not essential for formation of the zinc insulin hexamer, but they do affect its solubility and its

ability to crystallise. Pig insulin molecules pack in the crystals with contacts between two equivalent isoleucine A10 residues. In bovine insulin the A10 residues are valines. As a consequence the crystal packing of the hexamers is slightly different as is shown by a 2.3 Å difference electron density map. The faces of bovine insulin crystals grow at different rates from those of pig insulin crystals and thus the crystals have a rather different appearance (Fig. 3). Such changes of solubility and crystal form may affect granule formation; although the changes are only small they may reflect the differences in granules of different mammals and these may have functional significance. We cannot rule out the possibility that the nature of B30 is important to a species-specific proinsulin conversion enzyme, but it does seem that the changes at A8–A10 and at B30 found in mammals have little effect on the receptor binding.

Rats show polymorphism in their insulins, and rat insulin I is unique in that it contains a proline at B9, an otherwise invariant residue. The two rat insulins have similar biological activities. The rat I insulin, however, has a rather different dependence of its circular dichroism on zinc concentration (T.L.B., S.P.W., and A. Wollmer, unpublished). This indicates that the proline at B9 may decrease the association constant to zinc hexamers. Thus, this change at B9 may not be selectively neutral in terms of aggregation of insulin in the storage process, although it seems that given sufficient zinc, rat I like rat II insulin can crystallise as zinc hexamers. The packing relationships of the hexamers in crystals show very different symmetries for the two insulins. Rat I insulin crystals are rhombohedral whereas rat II insulin hexamers pack in a cubic lattice, space group $P4_332$ (Fig. 3). This cubic crystal form may be related to the unusually clear crystalline arrangement of some rat granules shown by electron microscopy.

For fishes the nature and location of the changes is different and certainly not random. The residues A8, A9 and A10 are very different from those found in mammals and the variation within fishes is much less. In particular A8, A9 and A10 are conserved in fishes as histidine-basic proline residues; in mammals they are smaller, non-basic and sometimes hydrophobic. In contrast to the mammals, residues A12–A15, A17 and B1–B3 are changed in fishes. Most also possess an extra B-chain residue at the amino terminus. These two groups of residues (especially B0, B1, A13, A14, A17) are not randomly distributed but are close together in the monomer and are clustered with equivalent residues when dimers aggregate to hexamers. The changes are complementary in that they still allow zinc hexamer formation. A single change of the kind observed in most of these locations would not favour hexamerisation. Although collectively these changes do not affect hexamer formation, they do affect the nature of the crystal packing and therefore perhaps granulation. In the laboratory cod insulin can be crystallised in conditions used to prepare pig insulin crystals but the crystal form is orthorhombic instead of rhombohedral, although the crystals also contain zinc insulin hexamers¹⁹. The sequence changes seen in the insulin of the jawless hagfish, however, do affect aggregation. In this case the zinc binding residue B10 is not histidine and the insulin does not form zinc insulin hexamers. The storage form must be radically different, although there may be some aggregation through non-polar surfaces²⁰. The hagfish insulin may resemble an ancestral insulin²¹ which was not subject to severe storage restraints and thus had not evolved the complex enzyme-resistant, storage mechanism typical of mammals.

The insulins of rodents from the suborder Hystricomorpha are different from the insulins discussed above as they do not show the uniform rate of evolution characteristic of the other insulins. The insulins of the guinea pig and coypu have changed at B10. The residue is no longer the zinc-binding histidine, but rather asparagine or gluta-

mine. Ultracentrifuge²² and circular dichroism²³ studies indicate that guinea pig insulin does not hexamerise in the presence of zinc. Examination of the changes in amino acid sequences of coypu and guinea pig insulins show that there are further changes related to this lack of association. Residues B14, B17 and B20 uniquely change in guinea pig and coypu insulins to larger and more hydrophilic residues. Residues B4 and A1 also change to radically different residues; they are larger and more basic. All of these residues are clustered together in the three-dimensional structure and make up the large surface which in other mammals is hydrophobic and involved in close contacts in the zinc insulin hexamer. The residue changes in the guinea pig and coypu are not only non-randomly located, they also make the surface more hydrophilic and thus the insulin molecule more thermodynamically stable in the absence of aggregation in aqueous environments. The relevance of this to storage is emphasised by the finding that guinea pig granules contain no zinc and do not have a crystalline appearance. Both guinea pig and coypu insulins also have changes in the B-chain carboxy terminus. In guinea pig insulin the change is at B22 (arginine to glutamic acid). B22 arginine is normally involved in an iron pair interaction with the carboxy terminus of the A chain. In bovine insulin, removal of the carboxy terminus by the action of carboxypeptidase destroys this stabilising ion pair; the tertiary structure is very disturbed and the insulin no longer dimerises. In a similar way, circular dichroism shows that guinea pig insulin has a changed tertiary structure and cannot dimerise²¹ (Fig. 4). Furthermore, guinea pig insulin has a very low biological activity, about 2% of that of bovine insulin in *in vitro* assays.

Evolution of insulins

These observations allow us to construct some hypotheses concerning the evolution of insulins. We will first consider guinea pig insulin. Let us suppose that there was a local shortage of zinc to the B cell in guinea pig ancestors. Insulin would not be aggregated with the same ease to zinc insulin hexamers. It would then be selectively advantageous to allow mutations which make the surface involved in aggregation to hexamers more hydrophilic to increase

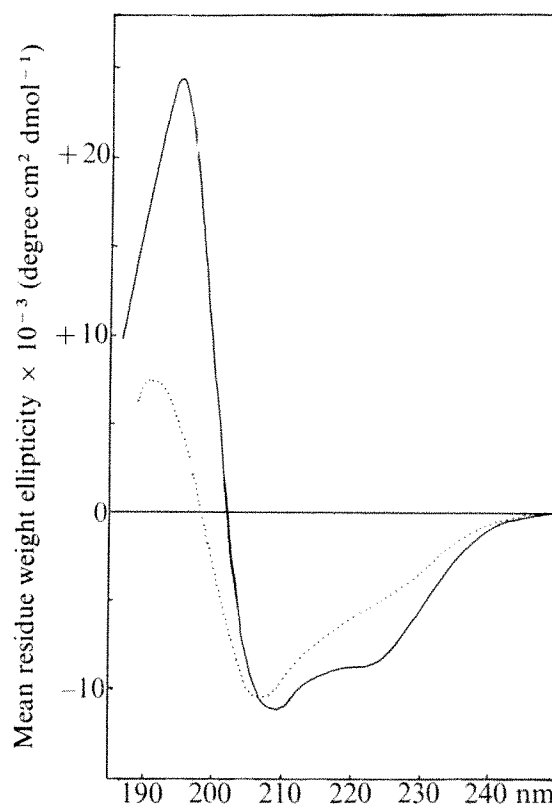


Fig. 4 Far ultraviolet circular dichroism spectra of bovine(—) and guinea pig (·····) insulin showing considerable differences in solution conformation.

thermodynamic stability. Although thermodynamic stability is increased, however, the unaggregated insulin molecules are still more susceptible to enzymatic degradation than zinc hexamers. It is well established that zinc insulin hexamers are much more resistant to tryptic digestion than the less associated forms. Thus it may be that the change of the trypsin-sensitive arginine at B22 to glutamic acid is selectively advantageous in terms of stabilising the molecule

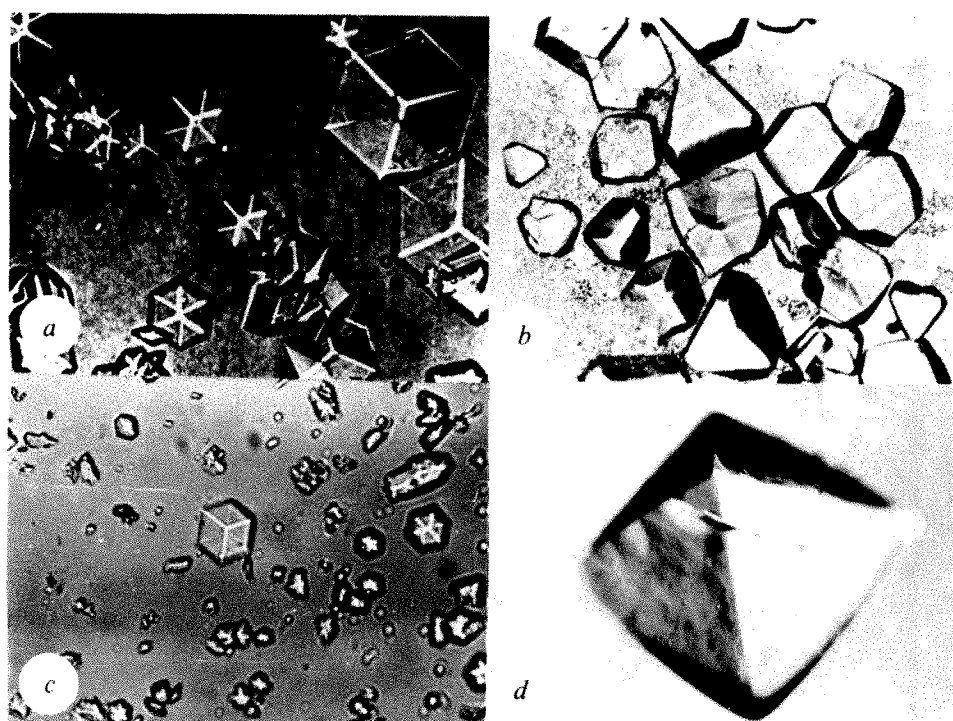


Fig. 3 Two Zn insulin crystals. a, Bovine; b, porcine; c, rat I; d, rat II.

to enzymatic degradation²². The changes, however, cause a change of tertiary structure, and this no doubt largely contributes to the decrease in binding affinity of the guinea pig insulin to adipocyte receptors. In fact it has been established that guinea pig insulin is less active even in guinea pigs compared with bovine insulin⁶. This implies that guinea pig insulin has evolved to maximise its stability in storage but this has been deleterious as far as receptor binding is concerned. We have to assume that the role of storage is as important to the fitness of the organism to survive as is the primary function, that of receptor binding and biological activity. In summary the arguments concerning the guinea pig insulin seem to implicate adaptive changes related not to receptor binding but rather to several other characteristics which are important to the physiological role of the insulin molecule. This is consistent with the high rate of acceptance of mutations in this and possibly some related species²⁴.

For other insulins the rate is less but uniform and this has been said to be evidence in favour of neutral mutations. Neutral mutations would be expected to occur randomly within certain areas of the molecule which are not affected by restraints. Our description of the location of the amino acid changes, however, has demonstrated that the location of accepted mutations has been distinctly different in different classes of animals. We have shown that these changes affect different physical properties such as three-dimensional structure, solubility, thermodynamic stability, resistance to enzymatic degradation, self-association and crystallisation to different extents. Furthermore, these changes will probably affect various aspects of the physiology such as activation of the proinsulin molecule, the nature of the storage of insulin in granules and its transport to the storage site and to the receptor apart from any effect on the receptor binding. It is therefore possible that they may also have been adaptive changes. Although the general nature of the insulin receptor binding has been retained during evolution, the nature of these other physiological restraints may have changed at different times. But how does this give rise to the apparent constant rate of amino acid substitution?

If there are a number of different restraints on the acceptance of mutations, a change of restraint will necessarily give rise to a noticeable change in the rate of acceptance in the following circumstances. First, if a change of restraint is very rare and occurs at only one or two points in evolution. Second, if changes in the insulin molecule are advantageous in terms of one characteristic but lead to a decrease in fitness in terms of other characteristics. Third, if by chance the restraints all changed in one phase of evolution due to changes in selective pressure on the organism as a whole. In the case of insulin, let us assume that adaptive changes dominate observed changes in evolution, but that the nature of the restraints has been different over different periods of time during evolution. This would be consistent with the several different locations of accepted mutations in insulins from different classes of animal. In the case of guinea pig insulin, the restraints are not independent in our model. Certain of the amino acid changes are involved in more than one aspect of function. Inability to bind zinc affects thermodynamic stability, susceptibility to enzymatic degradation and receptor binding of the insulin. Thus the restraints are interdependent and further mutations beneficially affecting each of these restraints become selectively advantageous. This gives rise to a cascade of evolutionary change.

In the other insulins we have no reason to suspect that changes which are adaptive in terms of one characteristic have serious consequences for other aspects of the physiology of insulin. Thus a change in the residues affecting crystallisation may be advantageous in terms of stability of the granules or the rate that they are dissolved in the circula-

tion, but it may not seriously affect the rate of activation of the proinsulin molecule, the receptor binding or thermodynamic stability. Thus the changes in restraint do not usually lead to a cascade effect, and they may be considered as independent variables. If the changes of restraint are frequent in evolution but independent, there is no reason why we should observe a sudden change in the rate of evolution. Statistically it is not unlikely that the changes of restraints (if there are a large enough number of them) will give rise to a fairly uniform rate of change in amino acid sequence, although more detailed examination of the changes would reveal their location in the protein not to be random. This is what is observed for most insulins.

This argument assumes that the total pressure on the molecule at any one time is roughly constant. This at first seems to be an unlikely assumption. But, if the restraint changes which give rise to adaptive mutations do not directly relate to major aspects of the physiology of the animals, then this assumption seems more reasonable. For instance, changes in the solubility of the granules and the stability of the insulin molecules in circulation may be related to rather sophisticated changes in the speed of response to insulin and to the time during which an insulin molecule is active before it is degraded and removed from the system. Changes of this nature in quite closely related species are not unreasonable and may increase the fitness of the animal to its environment. Furthermore, some adaptive changes may occur to eliminate undesirable situations such as chance interaction of the insulin molecule with other proteins within the cell and during circulation.

In the insulin molecule we have seen that the receptor binding is a fairly constant restraint during evolution, and this has led to an invariance of some surface residues and those residues in the hydrophobic core which are responsible for the general tertiary structure and consequently the proper arrangement of the residues binding the receptor. The number, nature and relative importance of restraints will differ from protein to protein and so the average rate of acceptance of mutation would be expected to vary although in many cases it might be relatively constant within a group of functionally equivalent proteins. For instance, in the proinsulin-connecting peptide most residues must be on the surface of the proinsulin molecule and therefore they will be more susceptible to variations in the environment of the protein. The connecting peptide must also accommodate changes in the insulin molecule whose surface it covers. It is therefore plausible that the characteristics which act as restraints on the acceptance of mutations change more frequently for the connecting peptide. This is consistent with the higher rate of change of this peptide than for the insulin molecule as a whole.

In conclusion, it seems that in rare cases an amino acid may be changed in a way that is advantageous to one characteristic but deleterious to others. The functional characteristics are interdependent and the rate of evolution will undergo a sudden burst. A uniform rate for most proteins is, however, not unexpected if the functional restraints are independent in terms of the amino acids which are affected. A uniform rate of evolution and the observation that amino acid changes are functionally insignificant with respect to one characteristic do not seem to be conclusive arguments for attributing most sequence variation observed in proteins to selectively neutral or non-Darwinian evolution.

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letters to nature

Precession in transient X-ray sources

THE origin of both the relatively long period and the transient nature of the recently discovered X-ray sources Ariel 1118-61 (ref. 1) and Ariel 0535+26 (ref. 2), with pulse periods of about 405 and 104 s, respectively, and decay times of days, is unclear. The pulse rate of A1118-61 has been attributed to an extremely short binary star orbital period³, or to an extremely long neutron star rotation period⁴. The quasi-sinusoidal nature of both X-ray light curves, unlike the sharply pulsed flux from Her X-1 and Cen X-3, would seem difficult to account for in a rotation model. The 104-s period, on the other hand, seems embarrassingly short for a binary orbital period. Therefore, I reiterate here an earlier suggestion⁵ that free precession may be important in such galactic X-ray sources. Such precession, which provides a plausible explanation for the 35-d quasi-sinusoidal period and intensity variation of Her X-1 (which has a 1.25-s pulse period) could directly account for the shape of the light curves of the transient X-ray sources.

If, as has been suggested, the initial X-ray outburst is triggered by the close approach of a neutron star (or white dwarf) to its main sequence companion, either because the collapsed star is moving in a highly elliptical orbit⁶, or because the radius of the companion is variable⁷, the accompanying rapid mass transfer might well provide the torque necessary to excite free precession of large amplitude in the collapsed object. For a neutron star of mass M , surface radius R , Alfvén surface radius r and total mass accreted m , the spin axis could change direction by an angle $\theta = mr^2/MR^2$ during a single encounter. The superposition of the effect of many mass transfer events (even if it is stochastic) could easily lead to a precession angle of large amplitude ($\theta \approx 10$ - 100°), since the damping times can be long (for example, greater than 10^6 yr for neutron stars⁸).

For a star spinning with a period T_s , and having matter density ρ , the free precession period T_f is roughly $T_f \approx \alpha T_s^3 G \rho$, where α is a numerical coefficient of order unity which depends on the detailed structure of the collapsed object (density distribution, rigidity, and so on). Identifying the observed periods of a few hundred seconds with the free precession period of a neutron star ($\rho \approx 2 \times 10^{14}$ g cm⁻³) and taking $1 \gtrsim \alpha \gtrsim 10^{-2}$, one finds $T_s \approx 0.1$ - 0.01 s. This range of spin periods is plausible, encompassing as it does the periods of three known pulsars (the Crab, binary and Vela pulsars with pulse periods of 0.033, 0.059, and 0.089 s, respectively). Such a high spin rate need not suppress matter accretion from a disk, provided that the magnetic fields of the neutron stars are somewhat smaller than the usually assumed value of 10^{12} gauss. The discovery of regular short-period pulsations

or amplitude variations in the X-ray flux from these transient sources would offer strong support for the present suggestion; its absence, although difficult to reconcile with most plausible beam profiles, is not decisive.

White dwarf X-ray sources, with spin periods of order 100 s, on the other hand, could also show evidence of free precession, but with a period of days. One might expect such a period to show up in the mean (averaged) intensity variation over an observation period of weeks. (I note that at least one white dwarf showing circular polarisation and thus probably having a strong surface magnetic field, G195-19, shows regularly periodic variations in polarisation with a period of 1.33 d (ref. 9), a period the discoverers attribute to rotation but which could well be the free precession period arising from a spin period of about 100 s.) In the present context, the discovery of either short (0.1-s) or long (1-d) periods in any of these X-ray sources could distinguish between models involving white dwarfs and those involving neutron stars.

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Possible cosmological origin of spontaneous symmetry breaking

POPULAR unified theories of weak and electromagnetic interactions¹ are based on the notion of a spontaneously broken gauge symmetry. The hope has also been expressed by several authors that suitable generalisations of such theories may account for strong interactions as well. Here we propose that the spontaneous breakdown of gauge symmetries may have a cosmological origin. As a consequence we find that at some early stage of development of an expanding universe, a phase transition takes place. Before the phase transition, weak and

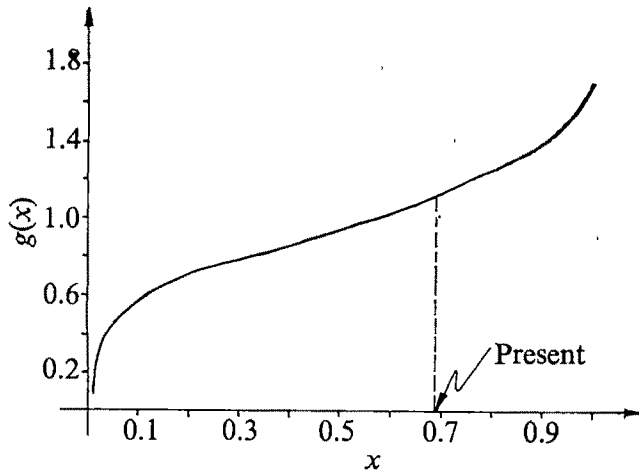


Fig. 1 Vacuum expectation value of the Higgs field plotted against the expansion parameter of a "standard" universe.

electromagnetic interactions (and perhaps strong interactions too) are of comparable strengths. The presently observed differences in the strengths of the various interactions develop only after the phase transition takes place.

In order to illustrate our ideas, we consider a 'proto-type gauge theory' of the Salam-Ward-Weinberg variety (with gauge group $SU(2)_{\text{LEFT}} \otimes U(1)_{\text{RIGHT}}$) in a curved space with prescribed metric tensor $g_{\mu\nu}(x)$. The action functional of the theory may be written as $W = W_0 + W_H$, where W_0 stands for the gauge-invariant action of massless Fermion fields and of the gauge fields. The form of W_0 is well known in flat space-time¹ and it is a trivial exercise to generalise that expression to a space of any given metric tensor $g_{\mu\nu}$. The action W_0 is automatically invariant under (infinitesimal) Weyl transformations, $\delta g_{\mu\nu}(x) = 2\Lambda(x)g_{\mu\nu}(x)$, $\Lambda(x)$ being an arbitrary smooth function. Likewise, the action of the isodoublet Higgs field, W_H , can be written in a form which is invariant under Weyl transformations^{2,3}:

$$W_H = \int d^4x \sqrt{(-g)} \left\{ -\frac{1}{2} g^{\mu\nu} \Phi^\dagger \left(\partial_\mu + i \frac{g'}{2} B_\mu + i \frac{g}{2} \tau A_\mu \right) \Phi \right. \\ \left. \times \left(\partial_\nu - i \frac{g'}{2} B_\nu - i \frac{g}{2} \tau A_\nu \right) \Phi - \frac{1}{12} R(\Phi^\dagger \Phi) - \frac{\lambda}{24} (\Phi^\dagger \Phi)^2 \right\} \quad (1)$$

Unless stated otherwise, we follow the notation of ref. 1 with obvious modifications to accommodate an arbitrary space-time metric. The quantity R stands for the Ricci scalar, $R = g^{\mu\nu} R_{\mu\nu}$. So Weyl (conformal) invariance can be broken only by the properties of the "cosmic background". In this approximation, our model is consistent with Mach's principle.

We assume that the metric is of a Robertson-Walker form,

$$ds^2 = dt^2 - a(t)^2 [(1-r^2)^{-1} dr^2 + r^2 d\Omega^2]$$

corresponding to a "standard" cosmology⁴, t being the "cosmic time", and so $a(t)$ is the radius of the universe. Units are chosen such that $\hbar = c = L = 1$ where L stands for the Planck length. We re-emphasise: the influence of the matter fields on the metric is neglected in this model. This implies that the energy-momentum tensor of matter can be written as $T_{\mu\nu} = p g_{\mu\nu} + (p + \rho) u_\mu u_\nu$, where p, ρ, u_μ are the classical pressure, mass-energy density and four-velocity, respectively.

Spontaneous symmetry breaking develops if the vacuum expectation value (VEV) of the neutral Higgs field, $\phi = \frac{1}{2} \times (1 - \tau_3) \langle \Phi \rangle$ differs from zero. In analogy with the flat-space case, ϕ satisfies the equation $(\square + (R/6))\phi + (\phi^3 \lambda/6) = 0$. To qualify as the VEV of a quantised field, ϕ can at most depend

on the cosmic time, t . Using the classical equations of the standard cosmology, $da = [(8\pi a^2/3) - 1]^{1/2} dt$, one introduces the expansion parameter $x = a(t)/a_{\text{max}}$ as an independent variable. The dimensionless parameter λ can be 'scaled out' by introducing the function $g = (\lambda a_{\text{max}}^2)^{1/2} \phi$. In the spirit of the standard cosmology, we assume that for $x \leq x_0$ the "matter" distribution is extremely relativistic ($p = (\rho/3) \approx 0$), while for $x \geq x_0$ the distribution is "pressureless", $p \approx 0$, $\rho \approx x^{-3}$. Thus, x_0 stands for the expansion parameter at which the "big bang" universe cools down sufficiently so as to become a "quiet" universe following a "fireball" stage. The value of x_0 is generally estimated to lie between the limits $10^{-6} \leq x_0 \leq 10^{-3}$, whereas the "present" value of x is approximately $x_1 \approx 0.69$. Corresponding to $p = \rho/3 \approx 0$ ($x < x_0$), and $p \approx 0, \rho \approx x^{-3}$ ($x > x_0$), we deduce,

$$\left(\frac{1-x^2}{x^2} \right)^{1/2} \frac{d}{dx} \left[x^3 \left(\frac{1-x^2}{x^2} \right)^{1/2} \frac{dg}{dx} \right] + \frac{x^3}{6} g^3 = 0 \quad (x < x_0)$$

$$\left(\frac{1-x}{x} \right)^{1/2} \frac{d}{dx} \left[x^3 \left(\frac{1-x}{x} \right)^{1/2} \frac{dg}{dx} \right] - \frac{1}{2} g + \frac{x^3}{6} g^3 = 0 \quad (x > x_0) \quad (2)$$

since, as a consequence of the Einstein equations, $R = (p - (\rho/3))$. The vacuum energy of the Higgs field is minimised by the trivial solution, $g = \phi = 0$ for $x < x_0$. In order to find the stable vacuum for $x > x_0$, we notice that by introducing the variable $y = (1-x)^{1/2}$ equation (2) for $x \approx 1$ (near maximal expansion) becomes

$$\frac{d^2 g(y)}{dy^2} - 2[g(y) - \frac{1}{3} g(y)^3] = 0 \quad (3)$$

In analogy with the flat space case, the stable solution of equation (3) is found to be $|g| \approx \sqrt{3}$ ($y \approx 0$). Thus, the VEV of the neutral Higgs field is zero as long as the universe is "hot" ($x < x_0$) and then it grows gradually to the limiting value, $\phi_{\text{max}} = (\lambda a_{\text{max}}^2/3)^{-1/2}$ as the universe cools down ($x > x_0$). In this model, the value of λ can be adjusted to reproduce the presently observed ratio of weak and electromagnetic couplings.

Equation (2) can be integrated numerically for $x > x_0$. A typical solution (with $x_0 = 10^{-4}$) is plotted in Fig. 1. We found that the solution for $x \geq 10^{-2}$ is very insensitive to the choice of x_0 within the above mentioned interval. Since $x_0 \ll 1$, the behaviour of $g(x)$ near x_0 can be estimated on the basis of the asymptotic solution near zero. There one finds $g(x) \sim x^\beta$ ($x \rightarrow 0^+$), $\beta = (\sqrt{17} - 3)/4 \approx 0.28$. Thus we approximate $\phi(x) \approx (x - x_0)^\beta$ near x_0 ; this behaviour is borne out by the numerical solution. Together with the relation between temperature and expansion parameter, $T x^2 \approx \text{constant}$, we thus estimate the critical exponent of ϕ to be $\beta \approx 0.28$ with a critical temperature $T_c \gtrsim 10^5$ K.

Since the effective weak interaction constant, G_F , is proportional¹ to ϕ^{-2} , its value changes very little during the present age: $\dot{G}_F/G_F = -2 \dot{x} \log g / dx \lesssim -10^{-10} \text{ yr}^{-1}$. But it is remarkable that according to this model, weak and electromagnetic interactions were of comparable strength (and of long range) within the primordial fireball. The consequences of this result (particularly with regard to a neutrino background) are at present under investigation.

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A Megalithic observatory on Dartmoor

ASTRONOMICALLY aligned standing stones have been shown to occur at many sites dating from the late Neolithic and early Bronze Ages in North-west Europe. We have examined the stone rows at Merrivale on Dartmoor (SX553746) and conclude that they, together with associated stone rings and menhirs, form a solar and lunar observatory. An interesting feature of this site is that it apparently includes a calculating device for facilitating the prediction of lunar eclipses. Thom^{1,2} has suggested that certain stone settings which are themselves not astronomical alignments, notably four fan-shaped arrangements of stones in Caithness (Mid Clyth, Dirlot, Loch of Yarrows and Camster) and the well known groups at Carnac and Kermario, are in fact for this purpose. The Carnac alignments are complicated and difficult to interpret completely, the Caithness settings have been disturbed and many stones are missing; at Merrivale the stone rows have not been greatly damaged and the simple method of their use, though not identical to that proposed by Thom, lends support to his basic contention that techniques for lunar eclipse prediction were developed at that period.

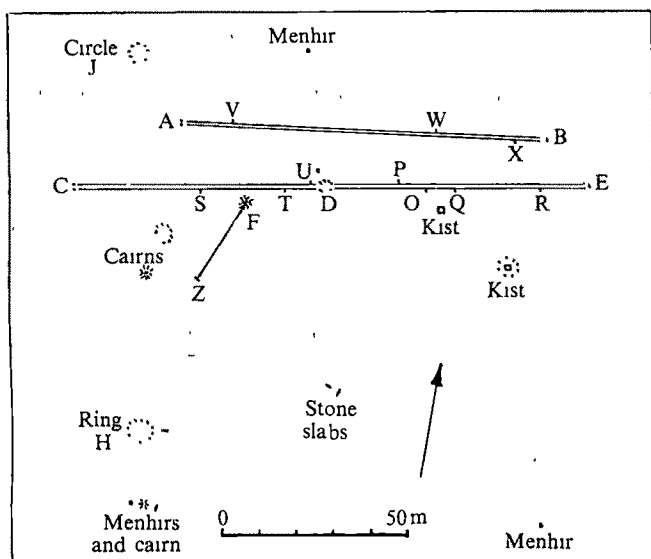


Fig. 1 The stone row site at Merrivale, Dartmoor.

We do not describe the Merrivale site in detail here, as it is hoped to publish a full account elsewhere. The stone rows (Fig. 1) lie on the summit of a fairly flat-topped bluff, which is covered with typical moorland vegetation of rough grass on peat and is, for the most part, free from boulders. There are two double rows, both roughly east-west; Row AB, 181.7 m long on an orientation of 83.7°, has 91 stones in its northern line, and 95 in its southern, most occurring in equally spaced pairs. Row CE has a length of 263.7 m, and is on an orientation of 81.7°. We found, with probing, 136 stones in the northern line and 130 in the southern. Neither double row is exactly straight, deviating about 2 m from the straight line, nor has a constant separation between the rows, which varies irregularly from 0.6 to 1.2 m. Both these rows have pairs of large stones to mark their western ends and a single stone set transversely to the rows at their eastern ends. Row AB has smaller stones than Row CE on average, but they are more uniformly spaced, with an average spacing of 1.86 m, whereas in Row CE the spacing changes from 2.05 m at the western end to 1.12 m at the eastern.

Row CE has a small egg-shaped ring, D, at about its mid point surrounding a cairn. Both rows have occasional large stones, (V, W, X, S, T, U, O, P, Q, R) along their lines, which stand about a metre high, compared with an average height of less than 0.5 m for the rest.

A third row of single stones, FZ 42.3 m, is at an angle of 57° to Row CE. The end of the row begins on the top of a small cairn and its first stone, F, is set with its broad face across the line of the row, as is the final stone Z. Very few stones are visible above the peat, and these are only about 0.1 m high. The row was probed and the peat was lifted to confirm the existence of possible stones. This row appears to be virtually complete, and there are 41 stones with a mean interval of 0.98 m. Apart from F and Z, none of the stones could have been more than 0.25 m above ground level, even in the Early Bronze Age, before the peat accumulation started.

About 100 m south of the rows is a well preserved ring H, of eleven stones (flattened circle, Thom Type B) and about 50 m north-west of A there is a true stone circle, J. The site also has several cairns, kists and four menhirs.

The site was surveyed by Lukis³ in 1879 and again by Worth⁴ who has also described some of its features⁵. During the investigation the site was resurveyed by sighting on the corners of three buildings whose 10-figure grid references were supplied by the Ordnance Survey Archaeological Division.

The north-west horizon, about 1.6 km from the site, is a smooth undulating moor with an elevation between 2.5° and 3.5°, broken by three separate outcrops of granite. Two of these outcrops make distinctive notches on the horizon. One of these, when viewed from the centre of ring H would have indicated the position of midsummer sunset (declination ϵ) in late Neolithic times, a second notch could have marked the position of the Moon, setting with declination $\epsilon + i$ (where i is the inclination of the Moon's orbit to the ecliptic) when viewed from the centre of J (Fig. 2).

We have been unable to find any outliers to the two stone circles which explicitly indicate these distant foresights. But two stone slabs in the centre of the site indicate the notch L on Fig. 2 and an outlier to the "egg" at D indicates notch M.

Although the horizon in other directions has prominent features, none of these is in a position to enable astronomical alignments to be made in other quadrants. Furthermore, the rows themselves are not aligned with astronomical significance.

For the present purposes the Moon's movements in declination may be regarded as a sinusoidal oscillation of amplitude ϵ and period 27.32 d, sinusoidally modulated by an oscillation of amplitude i and period equal to the rotation of the lunar nodes, 18.61 yr, and a minor perturbation, Δ , of amplitude 0.15° and period equal to half an eclipse year, that is 173.3 d. Whenever Δ is a maximum, the moon is at an "eclipse danger period". Thom points out that if Megalithic astronomers could make measurements that were accurate enough to observe the minor perturbations, they would be in a position to predict the possibility of lunar eclipses⁶. In essence this can be done by moving one's observation position from moonset to moonset, so that it seems always to set behind a suitable distant notch. Markers on the ground would effectively indicate a scale of declination measurement.

We propose the stone rows at Merrivale were used in this way, and that their purpose is to provide a permanent scale of measurement. The suggested method of use is that the observer moved, at each lunar setting, to that position along the double rows where he would see the Moon set in exactly the same place behind notch M. He could then place a temporary marker between the stone rows to indicate where he stood, and markers placed at successive moonsets would readily reveal the day on which the Moon had set with maximum declination, because on that occasion the markers would be nearest to the western ends of the double rows. The small deviations of the stone rows from straight lines would not materially affect the results. The rows are of such a length as to cover a lunar declination range of 26.0° to 30.1°, which means that they could have been used

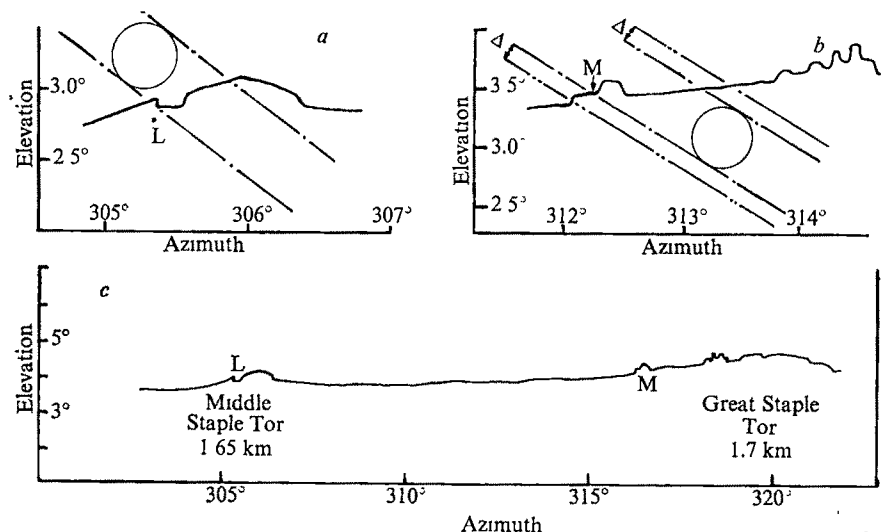


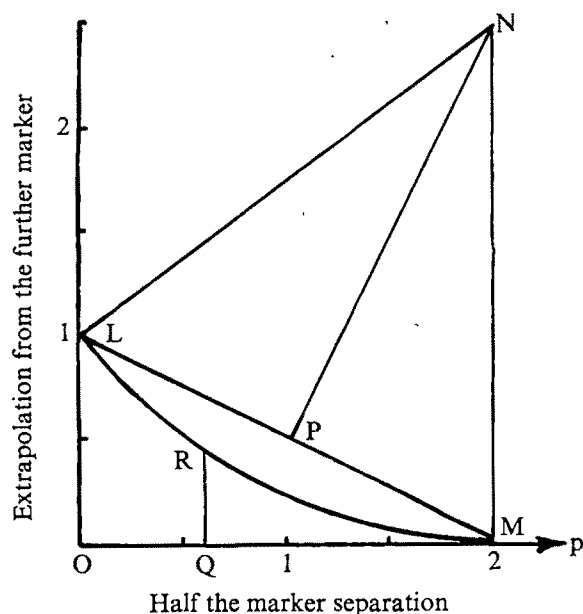
Fig. 2 *a*, The path of the setting Sun as seen from the centre of stone ring H in 2500 BC; *b*, the path of the setting Moon as seen from the centre of the stone circle J in 2500 BC; *c*, the north-west horizon as seen from the stone ring at Merrivale.

for about two years on either side of the declination maximum in the 18.61-yr cycle.

To achieve sufficient accuracy, the method needs to be elaborated, because the Moon may not be at its maximum monthly declination at the hour when it sets, and relatively small departures from the maximum would obscure the minor perturbation. Thom suggested a method by which the true monthly maximum declination could be obtained geometrically from the positions of markers placed on two successive nights. He shows that if the two markers nearest to the declination maximum are spaced a distance apart, $2p$, one must extrapolate a distance $G - p + (p^2/4G)$ from the further marker to find the position where one would have stood, if the Moon had set with maximum declination for the month⁷. G is a constant for the site; it is the ground equivalent to half a day's change in declination from the maximum, and its value could have been found empirically by the prehistoric astronomers.

Thom's method involves the fan-shaped stone settings referred to earlier, and gives a precise value for the extrapolation distance $G - p + (p^2/4G)$, but our method is to approximate the parabola given by Thom's expression by the arc of a circle.

Fig. 3 The curve LRM represents the extrapolation distance $G - p + (p^2/4G)$ as a function of p . It is approximated by the arc of a circle centred at N with radius $NM = 2\frac{1}{2}G$. Scales in units of G .



Thus on Fig. 3 a circle centred at N with radius $2\frac{1}{2}G$ very closely matches the parabola LRM. If this construction were to be laid out on the ground, then to find the required extrapolation distance, one would lay off a distance MQ equal to half the marker separation, and the perpendicular, QR, from the baseline to the arc would be the extrapolation. This geometric construction is not the only solution; the same extrapolation distance is obtained if the circle has a radius of $8\frac{1}{2}G$ and the whole of the distance between the markers is laid off along MO. In general, if a fraction r of the marker separation is laid off, the correct value for the radius of the circle is $\frac{1}{2}(1+16r^2)G$. This method of extrapolation appears to have been followed at Merrivale using the short stone row FZ.

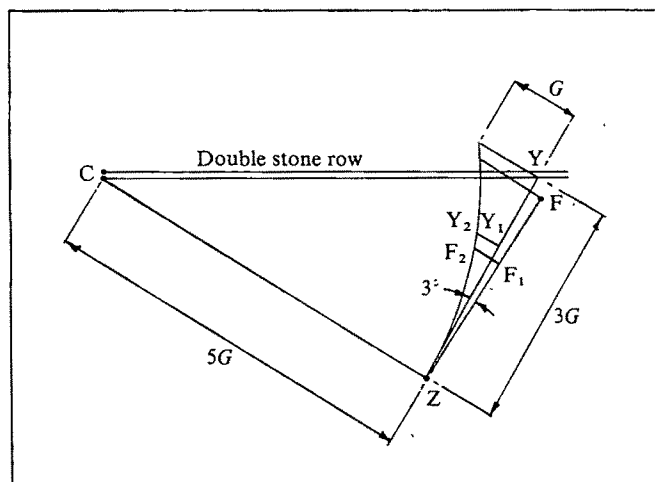


Fig. 4 The method of extrapolation at Merrivale. Lay off a distance FF_1 equal to $\frac{1}{2}\sqrt{2}$ of the marker separation; the extrapolation distance is the perpendicular F_1F_2 to the circle radius $5G$ centred at C. This gives a more accurate extrapolation than by laying off YY_1 equal to $\frac{1}{2}$ of the marker separation.

The Merrivale site has many interesting geometrical properties, including the repetition of certain units of length in its construction. One of these units is 15.6 m, which occurs as multiples along row CE in the spacings between the large stones. Thus we find (to within 1%) $OQ = 1$, $QR = 3$, $OR = 4$, $CU = 8$ and $UE = 9$ units. We are inclined to equate the unit of 15.6 m with Thom's constant G , which is calculated, using Heggie's method⁸, to have a mean value of 15.3 m over the site. The rows incorporate a triangle CZY (Fig. 4) in which ZY is the perpendicular to CZ, and if our interpretation is correct, two sides of the triangle, CZ (78.2 m) and ZY (45.6 m) are close to $5G$ and $3G$ respectively. This triangle ($r = \frac{3}{5}$) would therefore be one of the

family that could be used for extrapolation. The method would be to measure the distance between markers on successive evenings, lay off three-quarters of this distance from Y to Y_1 , and then project the perpendicular Y_1Y_2 to meet the circle centred at C. The length of this perpendicular is the distance to be added westwards from the further marker to get the position that the marker would have indicated, if the Moon had set with its monthly declination maximum.

In fact the short stone row FZ is not quite perpendicular to CZ and its length is 2.71G, not 3G. This slight modification to the simple scheme reduces the error inherent in this method of extrapolation, due to approximating the parabola with an arc of a circle, by about a factor of four and to a magnitude where it is insignificant. The distance to be laid out along FZ is not now $\frac{3}{4}$ of the marker separation, but $\frac{1}{2}\sqrt{2}$ which can easily be obtained by a simple geometrical construction using strings and pegs.

In examining prehistoric sites from an astronomical viewpoint one must guard against introducing fortuitous alignments or selective analysis of data. Thus one test for validity is the accuracy of the demonstrated alignments. Some authors, notably Thom, have taken great trouble with their surveys to find the exact declination of the Sun or Moon for the observing lines. In the present case it is difficult to apply this type of test, since the hypothesis is that the observers moved along the stone rows until they were observing the moonset exactly behind a particular distant feature. A test that can be applied is whether the hypothesis plausibly, and preferably quantitatively, explains the archaeological remains. The hypothesis advanced in this letter gives an explanation for the existence of the stone rows at Merrivale, including the precise dimensions and siting of the short single row, but there are many features of the site which we are unable to explain, of which the most obvious is why there are two double rows. For one apparently duplicates the function of the other. We have not examined any other site on Dartmoor.

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Palaeozoic secular variation recorded in Pendleside Limestone

RESULTS discussed here demonstrate the value of limestones in palaeomagnetic work. The fact that these rocks can apparently record secular variation provides evidence that the magnetisation is of depositional or very early diagenetic origin. Limestones may become a valuable tool in studying the ancient geomagnetic field.

Records of long term variations in the Earth's magnetic field over the past 15,000 yr or so come from lake sediments¹, marine sediments², archaeological materials³ and also from observatory records covering the past few hundred years.

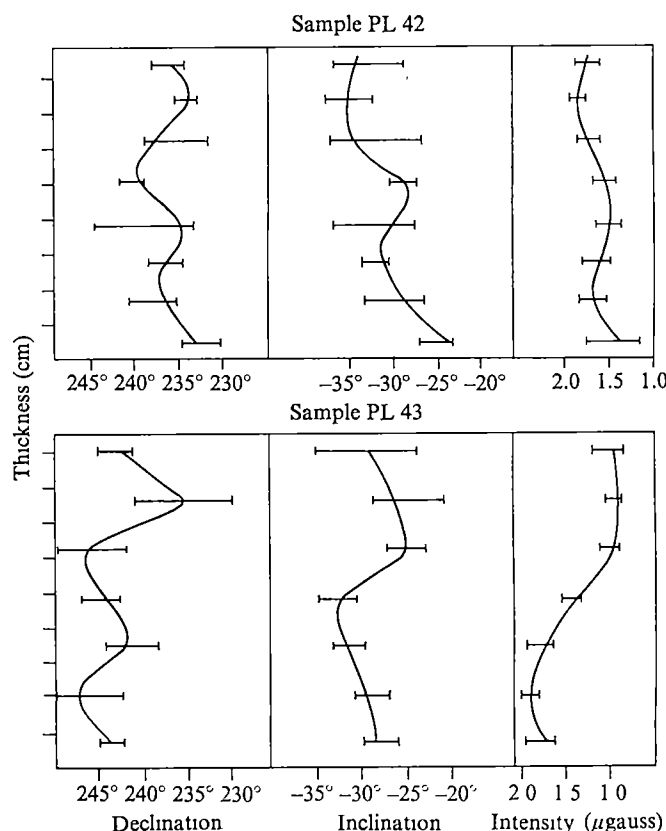


Fig. 1 The natural remanent magnetisation of two hand samples of Pendleside Limestone after a.c. cleaning at 150 oersted. Continuous curve is the mean of three curves obtained by slicing the cores into 1-cm disks. The error bars show the range of variation at each level.

Secular variations have also been convincingly demonstrated in Tertiary lavas⁴ and Mesozoic and Tertiary sediments. An important feature of secular variation is its strong dependence on latitude. Analyses of Recent geomagnetic data^{5,6} show that along lines of latitude progressively nearer the Equator, geomagnetic field directions become progressively more dispersed. Thus, the magnitude of secular variation should be greater nearer to the Equator. Tarling⁷, using the 1965 field data found a circular standard deviation ranging from about 7° near the poles to 19° near the Equator.

Although there have so far been no convincing records of Palaeozoic secular variation (before 225 Myr BP) variations could be preserved in certain Lower Carboniferous (Viséan) limestones⁸. The available evidence suggests that the magnetisation of these limestones, which is highly stable and easily measurable, was acquired during or very shortly after deposition.⁹

To test this hypothesis a large palaeomagnetic collection of over 50 sites (including a few hand samples) has been made. None of the results from the collection has been discounted and all will be reported elsewhere. Results from two hand samples (44 specimens) are presented here as they provide a convincing demonstration of secular variation in Palaeozoic limestones. The sampled section is in the upper part of the Worston Shale Group and the Pendleside Limestone at Salterforth railway cutting (OS Ref. SD 889459) in northern England. It consists of alternating dark, fine grained limestones and shales. The two hand samples discussed here were collected from different horizons 11 m apart in the Pendleside Limestone. Three cores were drilled perpendicular to the bedding plane in each sample and then sliced into seven or eight 1-cm disks which were measured using a Digico magnetometer. The disks were then cleaned in an a.c. field of 150 oersted and remeasured

(Fig. 1). Several factors suggest that the results record Carboniferous secular variation.

- The variation in declination and inclination approximates to a sine wave and is quite regular. The amplitude of the variations is comparable with other secular variation records. In view of the low palaeolatitude of the Pendleside Limestone (15°) it may be expected that the amplitude of the variations should be greater. There are, however, two reasons why the amplitude of the variations is anomalously low. In the first place, the Carboniferous geomagnetic field may have been much steadier than the Recent field. In the second place, and this seems much more likely, the smoothing may have been brought about by each specimen acquiring its magnetisation over a long period of time. Only if the magnetisation were acquired penecontemporaneously would the full amplitude of the secular variation cycle be preserved. The degree of smoothing shown by the curves shows that the magnetisation of each specimen partly represents an integration of contemporary geomagnetic fluctuations. This supports the contention that the magnetisation of the limestones is of early diagenetic origin⁹.

- The periodicity of the variation in declination is compatible with Recent records, assuming a sedimentation rate of 1 cm every 500 yr; such a sedimentation rate is quite consistent with our present knowledge of marine carbonate sedimentation rates.

- The validity of the variations is supported by the correlation between the three cores in each hand sample. Both declination and inclination plots are all in close agreement and only one measurement, in the declination of PL42 (Fig. 1), is slightly out of phase.

- Comparison between the declination and inclination curves indicates that the variation in inclination is less than that in declination (Fig. 1). This is generally the case for many secular variation records. Moreover, both curves show trends with two declination swings to one inclination swing. This is another feature common to many secular variation records.

- There is some agreement between inclination and intensity of magnetisation, such as may be expected were the variations really secular variations. Low-field susceptibility has been measured to check if the intensity variation is a function of magnetic mineral content. This is not the case because no variation in susceptibility (13.5 μ gauss oersted⁻¹) could be detected within or between cores.

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Earliest calcareous foraminifera

WE present here evidence that early Palaeozoic calcareous foraminifera existed and were relatively complex (multilocular and multiserial) in form but that they have been regarded formerly as calcareous algae. The early part of the invertebrate fossil record during the Cambrian period seems to show a striking contrast between the abundance of highly developed organisms such as the trilobite arthropods and the paucity of simpler forms such as the foraminiferal protozoans. Foraminifera are very small acellular animals with an important role in marine food chains¹ and it is reasonable to expect

them to have been a significant component of the benthic fauna throughout the Palaeozoic. Yet few foraminifera have been reported from the Lower Palaeozoic and those described from the Cambrian are mainly simple forms with unmineralised tectinous or agglutinated tests^{2–4}. Some more complex agglutinated forms are known from the Upper Cambrian⁵, but the first generally recognised calcareous foraminifer is the uniserial *Saccaminopsis* from the late Ordovician⁴. Consequently, the assumptions that Lower Palaeozoic foraminifera were primitive and mainly non-calcareous have been taken as bases for inferences concerning the early evolution of the group^{3,4} and the paradoxical, yet apparently inescapable, conclusion has been drawn that Cambrian foraminifera are very scarce.

We describe here small, septate chambered organisms with a thick calcareous wall belonging to a generic group centred on *Renalcis* Vologdin, and termed here renalcids. At least six nominal genera are involved (*Renalcis*, *Nubecularites* Maslov, *Chabakovia* Vologdin, *Shuguria* Antropov, *Izhella* Antropov, *Nephelostroma* Dangeard and Doré) although only two, *Renalcis* and *Izhella*, are likely to prove acceptable. Renalcids range throughout the Cambrian and are also well known from the Lower Ordovician and Upper Devonian. *Renalcis* has also been found recently in the Silurian of Khazakhstan, USSR (I. T. Zhuravleva, personal communication). They have a global distribution and are locally very abundant in a variety of early and middle Palaeozoic bioherms constructed by stromatolites, archaeocyathids, sponges and stromatoporeoids⁶. Various affinities, some foraminiferal but mostly algal (Table 1), have been attributed to renalcids. The view that they are algae is not consistent with their morphology and a more satisfactory explanation is that they belong to the Palaeozoic foraminiferal superfamily Parathuramminacea⁷.

Renalcids have a microgranular calcareous wall and a septate chambered structure. An individual test can include from 2 to more than 80 chambers. Internal chamber sizes range from 10×40 to 200×250 μ m. Wall thickness ranges from 5–125 μ m. The chambers are connected by intercameral foramina in the septa (Fig. 1). Apertures seem to be lateral and there are no pores in the wall. The complete test is generally between 500 μ m and 1 mm in size. These dimensions are based on numerous measurements of Cambrian and Devonian specimens. Chambers are usually arranged in several branching series. In random thin sections these seem to arise from a cluster of small chambers (Fig. 1) or from a large initial chamber which may be the proloculus. The specimen shown in Fig. 1 is from the Devonian and has been selected for its good state of preservation, but closely comparable specimens have been figured from the Cambrian of Siberia⁸. Renalcids may occur free in fine-grained sediment or attached to larger skeletons⁹.

Attributions of algal affinity to renalcids have been conflicting and are difficult to support. Both blue-green and red algae have been referred to. Blue-green algae are either filamentous or coccoid in form. Calcified filamentous blue-greens produce non-septate tubiform skeletons ascribed to genera such as *Girvanella* and *Ortonella*⁹. These are much smaller and totally

Table 1 Affinities formerly attributed to *Renalcis*, *Izhella* and their synonyms

	Blue-green alga	Red alga	Foraminifer
<i>Renalcis</i>	Korde ⁸ Johnson ²¹ Wray ²⁶	Korde ¹¹ Vologdin ²⁶	
<i>Nubecularites</i>	Maslov ¹⁰		
<i>Chabakovia</i>	Voronova ²⁷	Korde ⁸ Vologdin ²⁶	Elias ¹³ Klovan ¹¹ Antropov ¹⁵ Loeblich and Tappan ⁴
<i>Shuguria</i>			
<i>Izhella</i>	Antropov ²⁸		
<i>Nephelostroma</i>	Dangeard and Doré ²⁹		

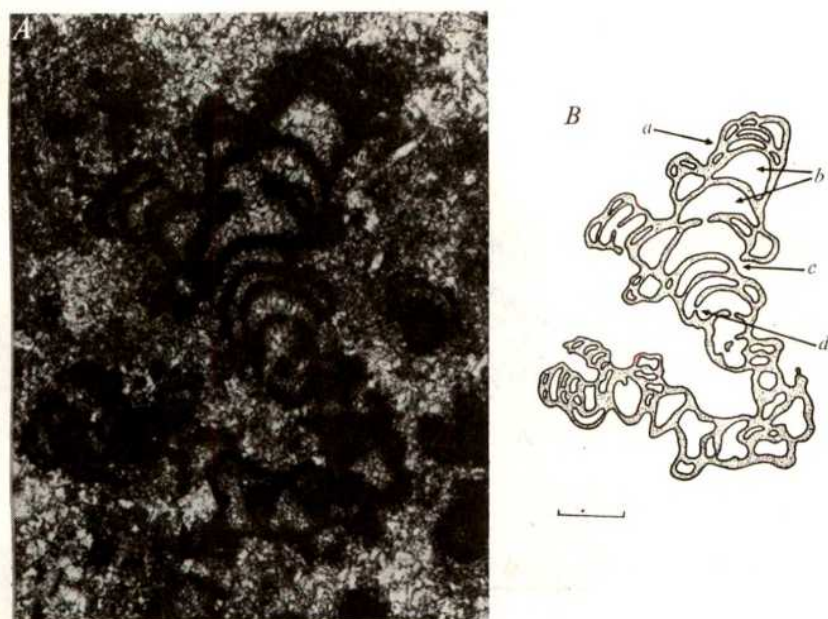


Fig. 1 Test of *Renalcis* sp. (Upper Devonian, Mount Hawk Fm., Mount Haultain, Alberta, Canada), showing branched multilocular form. A, Photomicrograph ($\times 50$); B, diagram indicating foraminiferal features: a, microgranular calcareous wall; b, chambers; c, lateral aperture; d, intercameral foramen; scale bar, 250 μm .

different in morphology from renalcids. Calcified coccoid blue-greens are not known from the Recent but calcification of the mucilaginous sheath surrounding coccoid cells could also be expected to produce skeletons morphologically and dimensionally quite distinct from renalcids. It is inconceivable that the chambers of renalcids represent individual blue-green cells, as Maslov¹⁰ has suggested, since their sizes differ by an order of magnitude. Korde^{8,11} suggested that the renalcid wall has a minutely cellular structure which indicates an affinity with red algae. But the cellules she reported are orders of magnitude smaller than the cells of undoubted fossil and Recent calcareous red algae. Further, we have been unable to detect cellular microstructure in the walls of renalcids from a variety of geological situations. Rather, the tests seem to comprise finely granular calcite with no visible structure, an observation supported by scanning electron microscope (SEM) studies¹².

The size and morphology of renalcids are, however, consistent with their being foraminifera. The skeletons consist of chambers, connected by intercameral foramina and with lateral apertures, arranged in extended branching series. A foraminiferal affinity for renalcids has received some previous support^{13,14} (Table 1). Even so, uncertainty has remained concerning their precise position. Elias¹³ thought that *Chabakovia* was related to *Ptychocladia* and placed both in a new foraminiferal family, *Ptychocladidae*. Loeblich and Tappan⁴ placed this family in the Endothyraea, but tentatively removed *Chabakovia* to the problematic group Reitlingerellida. They placed *Shuguria* in the parathuramminacean family Caligellidae. In our opinion the rather irregular form, relative morphological simplicity, and finely granular unlayered calcareous wall favour the placement of the renalcids as a discrete group in the Parathuramminacea⁷.

We propose to name this family Renalcidae, defined as follows. Test free or attached, irregular, branching uniserially; chambers numerous, lunulate to hemispherical, early chambers inflated; wall thick, calcareous, microgranular; apertures lateral. Type-genus *Renalcis* Vologdin. The Renalcidae differ from other parathuramminacean families in having thick walls, closely compressed chambers, and a tendency to branch. They most closely resemble the Caligellidae but differ in having a well developed septation, more compact form, and a lack of well defined coiling at any stage. The *Ptychocladidae* are very similar to the Renalcidae in the form of their branched uniserial test, but differ in their prostrate and always attached habit, radial partitions in the larger chambers, and layered wall.

The foraminiferal nature of renalcids has significant bearing on the early history of the Foraminifera: calcareous stocks now seem to be as ancient as agglutinated stocks, provided

that dubious Proterozoic specimens⁵ are disregarded. *Renalcis jacuticus* is one of the first calcareous organisms to appear in the lowest stage of the Cambrian, and the underlying Precambrian Yudomian strata have yielded *Nubecularites abustus*¹⁶.

In the Baltic region the agglutinated foraminifer *Platysolenites antiquissimus* (homeomorphic with *Bathysiphon*) is, likewise, one of the first shelled organisms to appear in the sequence, occurring in the Precambrian Vendian strata¹⁷. The Vendian and Yudomian are of similar age¹⁸ and the detailed biostratigraphy is uncertain, so that it is hard to say precisely which stock, calcareous or agglutinated, is the older. Consequently, it is difficult to support the inference that all calcareous foraminifera arose from agglutinated stocks as suggested by Loeblich and Tappan¹⁹. Both seem more likely to have arisen independently from simple, late Precambrian, tectinous forms.

With the recognition of renalcids as foraminifera two Cambrian foraminiferal biofacies now become apparent. Agglutinated microfaunas (for example, *Scaniella*, *Platysolenites*, *Psammospaera*, and *Hippocrepina*) dwelt in predominantly argillaceous sediments, associated with hyolithids, hyolithellids, trilobites, brachiopods, and siliceous sponges²⁰. Conditions were probably turbid and cool^{17,20}. Calcareous microfaunas (renalcids) dwelt in predominantly calcareous sediments associated with archaeocyathids, trilobites, chancellorids, gastropods, and the alga *Epiphyton*. Conditions were probably warm, clear and shallow²⁰. Warm, shallow-marine, carbonate environments of Upper Palaeozoic–Recent age also contain abundant calcareous foraminiferal assemblages. If renalcids were lacking it would be difficult to explain why similar habitats were unoccupied by calcareous foraminifera in the Lower Palaeozoic, especially as conditions favoured many other shelled organisms, and other not entirely hospitable environments supported foraminiferal assemblages.

The basic features of the renalcid test may be explained ecologically. Warm, CaCO_3 -saturated waters favour the secretion of thick calcareous walls, whereas agglutination is favoured in cooler or less saturated waters²¹. Chambers and septa, formed by periodic growth, serve as physical and osmotic barriers for the protoplasm inside²². Protection of this kind is of value in shallow-water biohermal environments where temperature, water-movement, predation, and ultraviolet radiation are likely to be high and where plant respiration and photosynthesis may cause local fluctuations in pH and Eh. Chambers may also help to culture algal endosymbionts, an association commonly found in shallow-marine tropical foraminifera²³.

We conclude that renalcids were the earliest calcareous foraminifera and belong to the superfamily Parathuraminacea. Their presence in the early Cambrian suggests that calcareous foraminifera arose, independently of agglutinated foraminifera, from tectinuous forms. Renalcids were adapted to the warm, shallow-marine, biohermal carbonate environments found widely across the continental shelves in early Palaeozoic times.

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Mechanism of oxidation of polyacrylonitrile fibres

THE first and most important stage of the preparation of high modulus carbon fibres from polyacrylonitrile (PAN) textile fibres is the oxidation of the fibres under tension. This produces an oxidised ladder polymer structure approximately parallel to the fibre axis which may be regarded as the template for the formation of the oriented carbon fibre. The exact structure of this oxidised polymer is not clear although four different ones have been proposed^{1–4}.

The structure of the unoxidised ladder polymer, which is formed without weight loss by heating PAN in a vacuum at temperatures of 180–230 °C is well established however, and, neglecting initiation points, is a condensed naphthyridine ring structure⁵:

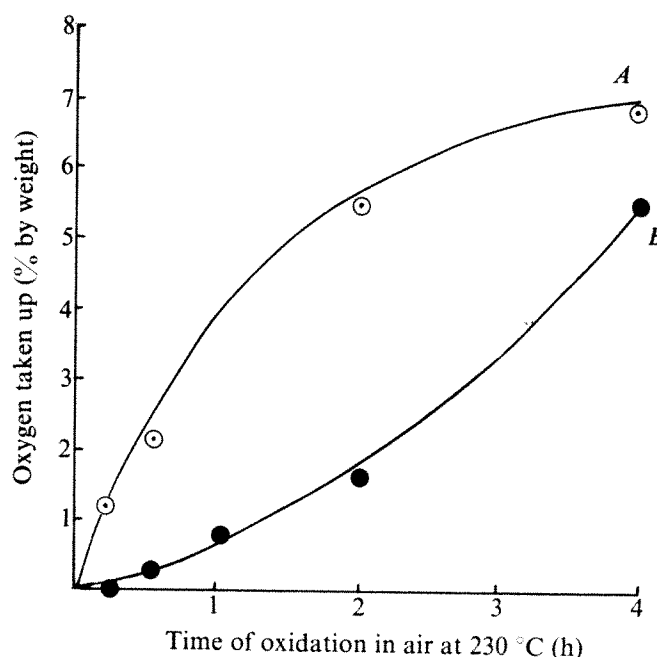
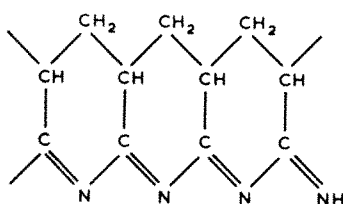


Fig. 1 Oxidation with no previous treatment. The values for oxygen taken up are the total found by analysis less the value before oxidation. A, Fibre A; B, fibre B.

This structure confers a copper colour to the fibres heated in vacuum because of the conjugated $\text{C}=\text{N}-\text{C}=\text{N}-$ system.

Textile PAN fibres are copolymers of acrylonitrile with one or more comonomers which are added to provide dye sites and also, by introducing points of structural inhomogeneity, to act as internal plasticisers and make fibre drawing easier. The various proprietary PAN fibres have different comonomers in them, a common quantity being about 6% by weight. It has not yet been shown, however, what effect these different comonomers have on the oxidation kinetics of PAN fibres. In this communication we compare the oxidation kinetics of two commercially available PAN fibres.

Fibre A was a round wet-spun fibre of 1.5 denier (cross-sectional area $133 \mu\text{m}^2$) of composition 95.0, 4.6 and 0.4 mol % of acrylonitrile, methyl acrylate, and a vinyl acidic compound providing COOH groups directly attached to the all-carbon polymer chain. Fibre B was dry spun with a 'dog-bone' cross section of 1.8 denier (cross-sectional area $165 \mu\text{m}^2$) and of composition 95.4 and 4.6 mol % of acrylonitrile and methyl acrylate, respectively.

The oxidation of the fibres was investigated by estimation of the oxygen absorbed after different oxidation times at 230 °C in air with the fibres tied on a glass frame and by examination with an optical microscope of cross sections of oxidised fibres cut to $1 \mu\text{m}$ thickness on an LKB microtome. The oxygen absorbed is shown in Fig. 1 and the cross sections of the fibres after 4 h of oxidation are shown in Fig. 2A and B. Fibre A has a fast initial oxidation rate and the sections showed what we have previously called an oxidation zone⁶ inasmuch as it moves inwards at a rate proportional to the square root of the oxidation time. Fibre B has a slow initial rate which increases with time, but at 230 °C does not show any oxidation zone, at least up to times of 4 h. The colour gradually changes uniformly over the section to a mid-brown.

As-received fibres were heat treated in a vacuum for 6 h at 230 °C, again while tied on glass frames. The weight changes were negligible but both groups of fibres were copper coloured, indicating extensive transformation into ladder polymer. The oxidation at 230 °C and microscopical examination of these fibres was carried out as before and results are shown in Fig. 2C and D and Fig. 3; the sections showed oxidation zones after only 1 h of oxidation. Clearly the vacuum heat treatment before oxidation has not changed the oxidation

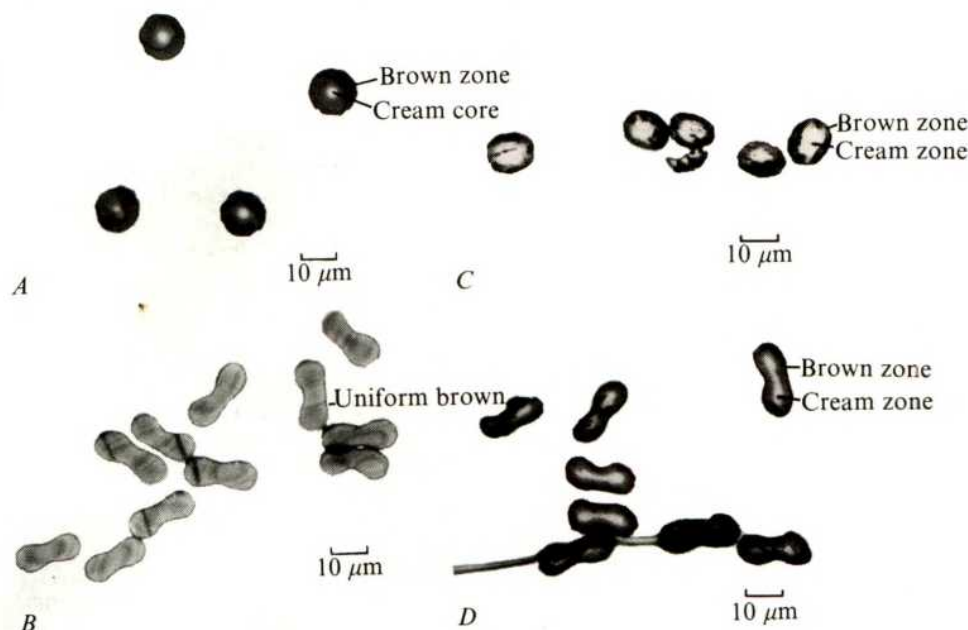
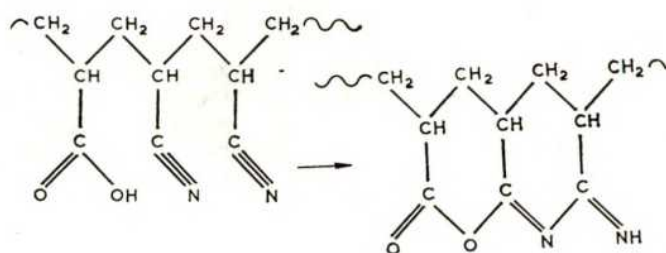


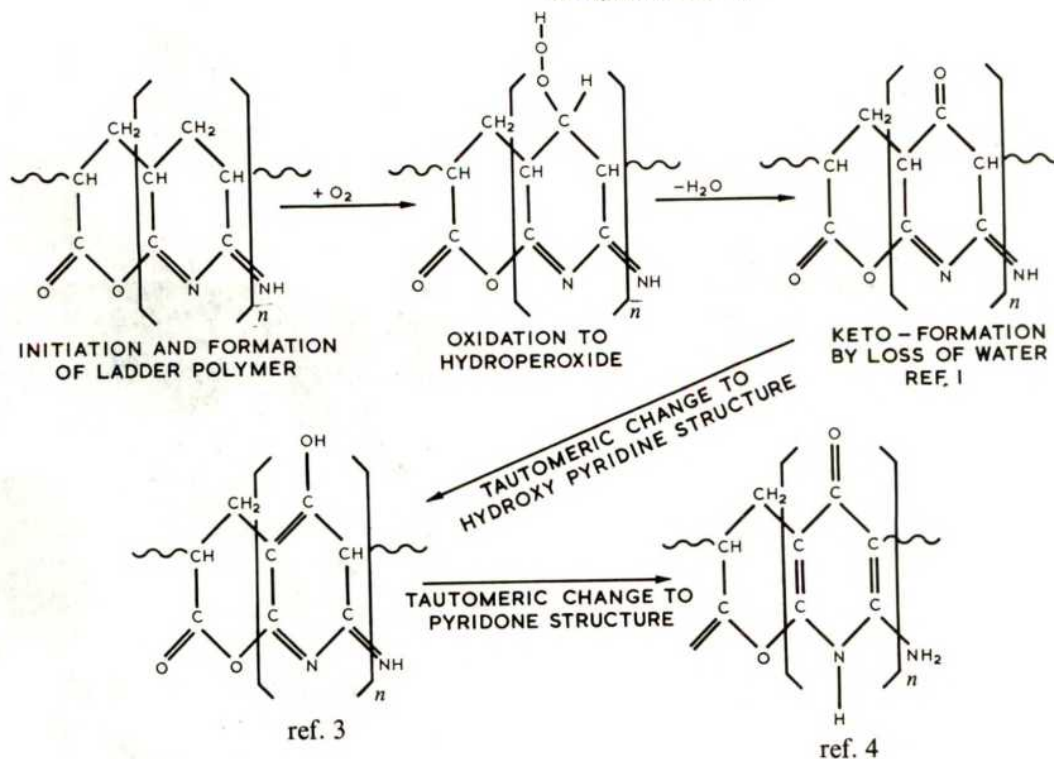
Fig. 2 A, B, Cross sections of fibres A and B, respectively, after 4 h in air at 230 °C. C, D, Cross sections of fibres A and B, respectively after 6 h in vacuum at 230 °C and 1 h in air at 230 °C.

kinetics of fibre A but has had a marked effect on fibre B, so that the oxidation kinetics are now similar to those of fibre A.

It seems that it is ladder polymer which is oxidised and this must be formed first. The acidic constituent of fibre A acts as an initiator for ladder polymer by the following mechanism as shown by Grassie, and this occurs rapidly at 230 °C:



Fibre B, with no acidic constituent, does not initiate ladder polymer formation very readily and this explains the different oxidation curves before and after the heat treatment. The presence of oxidation zones in fibre A, with or without heat treatment, shows that the rate of oxidation of the ladder polymer is faster than the rate of diffusion of oxygen in the fibre at 230 °C, whereas in untreated fibre B the rate of formation of ladder polymer and thus the rate of oxidation, is slower and cannot keep up with the rate of diffusion at 230 °C so that no oxidation zones can be seen unless the fibre is vacuum heat treated first. The conclusion drawn from these results, that formation of ladder polymer is a prerequisite to oxidation, is consistent with the structural formula for the oxidised ladder proposed by Potter and Scott⁴, based on the susceptibility to air oxidation of the ladder structure to give a pyridone structure. Three out of the four formulae proposed for the oxidised ladder polymer can, however, be reconciled if account is taken of oxidation mechanism and successive tautomeric changes, as follows:



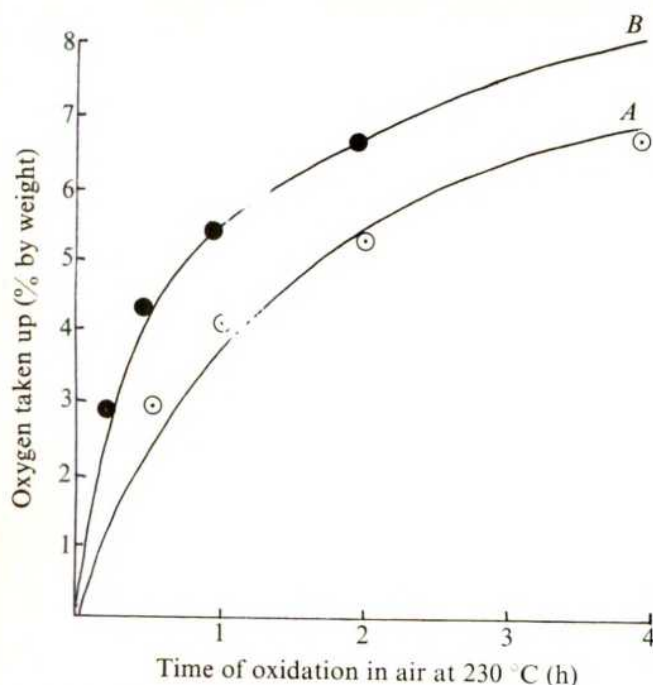


Fig. 3 Oxidation after 6 h of vacuum treatment at 230 °C. ○, Fibre A; ●, fibre B.

The lengths (n) of the conjugated sequences are probably about 4–5 monomer units and the formulae proposed apply only to the oxidised ladder polymer. No account has been taken of some chain scission which does occur during oxidation, as shown by the evolution of CO_2 (presumably from $-\text{COOH}$ groups) in the early stages of the subsequent inert pyrolysis to carbon fibres. These can be formed by oxidation of the polymer ends resulting from the scission, and these $-\text{COOH}$ groups thus formed can then initiate chain scission⁷. This may be the mode of initiation with fibre B, thus accounting for the acceleration of the oxidation rate with time.

The technique of examination of thin sections of oxidised fibres seems to be a new and sensitive technique of assessing PAN fibre oxidation kinetics and is being followed up in more detail. It is more positive than the examination of polished cross sections which we have previously used to investigate the oxidation of 3 denier ($284 \mu\text{m}^2$) PAN fibres⁶.

Our assumption that the outer zone was oxidised PAN whereas the inner core was not has been verified using an electron microprobe analyser tuned for oxygen detection and comparing the oxygen level in the zone and core of an oxidised fibre. It was found that the brown outer zone is relatively rich in oxygen compared with the light yellow core⁸.

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Artificially triggered lightning above land

LIGHTNING has been triggered artificially in the Massif Central by launching small rockets carrying a thin steel wire connected to the ground. During two investigations in 1973 and 1974, a total of 20 triggerings have been achieved. Photographic evidence establishes the existence of two kinds of luminous object, lasting several tenths of a second, which may be similar to some varieties of ball lightning.

A sudden perturbation of the electric field can trigger lightning^{1,2} and deliberate triggering of lightning with low cost equipment was first demonstrated by Newman *et al.*³, who launched small rockets carrying a thin wire connected to earth towards electrified clouds. The experiments were performed from a ship; our experiments extend this work to a fixed land-based station. Notwithstanding the lack of mobility, such a station does allow experiments that would be impossible or at least very difficult to perform at sea.

Our programme is the result of a joint effort by the Commissariat à l'Energie Atomique (CEA) and Electricité de France (EDF). The wire and rocket technique was developed initially at the CEA in the hope of perhaps shedding new light on the controversial subject of ball lightning⁴, since according to published statistics⁵ ball lightning is mentioned in more than 40% of the cases in which an observer describes a nearby lightning stroke.

The experimental lightning station was installed by EDF⁶ at Saint Privat d'Allier in the Massif Central, a region where the isokeraunic (thunderstorm) level is about 32. The area is rather flat locally, at an altitude of 1,100 m in the vicinity of smooth mountains which reach 1,300 m. The station consists of three main parts: the staff shelter, which contains the control desk and various cameras; the EDF tower; and the CEA range. An observation hut has been installed at a distance of 2.7 km from the main complex.

The EDF metal tower is 24 m high and is 110 m from the shelter. During the 1973 investigation, rockets were launched from a platform situated half way up the tower under a copper ring, 4 m in diameter, fixed to the top. It was expected that the metal wire carried through the ring by the rocket would make an electrical contact sufficient to conduct the lightning current to the top by way of the ring. All lightning, except one, however, struck the platform directly at the first stroke. When the flash comprised several strokes the subsequent ones were captured by the ring. Consequently, for the 1974 investigation the tower was modified for launching the rockets from its top. Four launchers were permanently loaded on the platform in 1973 and six on the top in 1974.

Fig. 1 Triggered lightning photographed from a distance of 2.7 km.



The CEA range was installed to provoke the interaction of lightning strokes and of lightning current with natural materials. The underlying idea is that some organic or powdery material is present in many instances when ball lightning occurs. This has been noticed already by some ball lightning theorists. So in our experiments lightning was also attracted to the ground in a meadow where two launchers were installed in 1973 and four launchers in 1974. First, experiments were performed with the wire reel laid on the ground. Later it was placed on a porcelain insulating pillar about 1 m high. This was tried in an attempt to delay fusion of the wire and to get bigger strokes. Finally, during the 1974 investigation, each reel was fixed to the top of a vertical pipe 1.5 m high and 14 cm in diameter, made of asbestos concrete and internally coated with soot. This tentative disposition was adopted to try to reproduce the creation of ball lightning when a chimney is struck by lightning. Measurement of peak lightning current is performed with magnetic link arrays, nine links being placed on wooden supports with threefold symmetry around each pipe.

The station is equipped with several devices for storm detection and electric field measurement^{7,8}. During storms the decision to fire a rocket depends on the indication of a field mill electrometer. We used standard anti-hail rockets (Ruggieri type 614) attached to a 0.2 mm steel wire unwinding off a reel. The reel is supplied with a cylindrical metal casing fitted with a conical nozzle and brake; it is sufficiently small ($h = 20$ cm, outer diameter = 5 cm) for easy fixing at the place where one wants the lightning to strike. The electrically fired rocket pulls the wire to a height of 700 m in about 5 s. Then it is automatically destroyed in accordance with safety regulations.

Between mid-July and the end of September 1973, 12 triggerings were obtained with 20 technically correct launchings. In 1974, eight triggerings resulted from 10 correct launchings between June 1 and October 15. Eleven flashes out of a total of 20 had at least one intensity peak greater than 2 kA, the highest value being 19 kA.

Triggered lightning seems to be similar to upward natural lightning striking tall structures^{9,10}. As a rule, triggered lightning begins with a slow discharge, the intensity of which remains in the kiloampere range, or less, with a rise time longer than 10 μ s and a total duration of a few tenths of a second. In nine cases the slow discharge was followed by several restrikes with steep-front, high current peaks of short duration.

In the case of negatively charged clouds, pictures taken from the distant observation hut show that branching may be very important (Fig. 1). On this photograph the vertical visible extension is of about 2 km and the total lateral extension at least 4 km. The overexposed part at the bottom corresponds to the 150-m length of vaporised wire. The shape of the branches is characteristic of upward discharges. This is confirmed by cine film (48 frames s^{-1}) which shows a rather low upward speed of about 2×10^4 m s^{-1} at the beginning. Subsequent strokes are correlated with the successive illumination of various branches in which the propagation is too fast for our measurements.

With positively charged clouds, triggering seems more difficult in the sense that the field at the ground has to be higher for the same probability of success. In this case the discharge also propagates upwards but with a greater speed of the order of 10^5 m s^{-1} or more; branches are also less numerous. Of course these conclusions are tentative since they are based on a small number of events.

We have not observed anything comparable with the more remarkable tales of ball lightning folklore. Nevertheless our search for ball lightning has not been fruitless. Several observations have been made which have a connection with the subject and which may help to explain some occurrences of ball lightning.

The beaded appearance of triggered lightning during its decay was noticed when triggering was unexpectedly produced by a plume of water ejected by a submarine explosion¹. In our experiments, the beaded decay has shown up systematically, and increasingly with increasing lightning strength. It has been

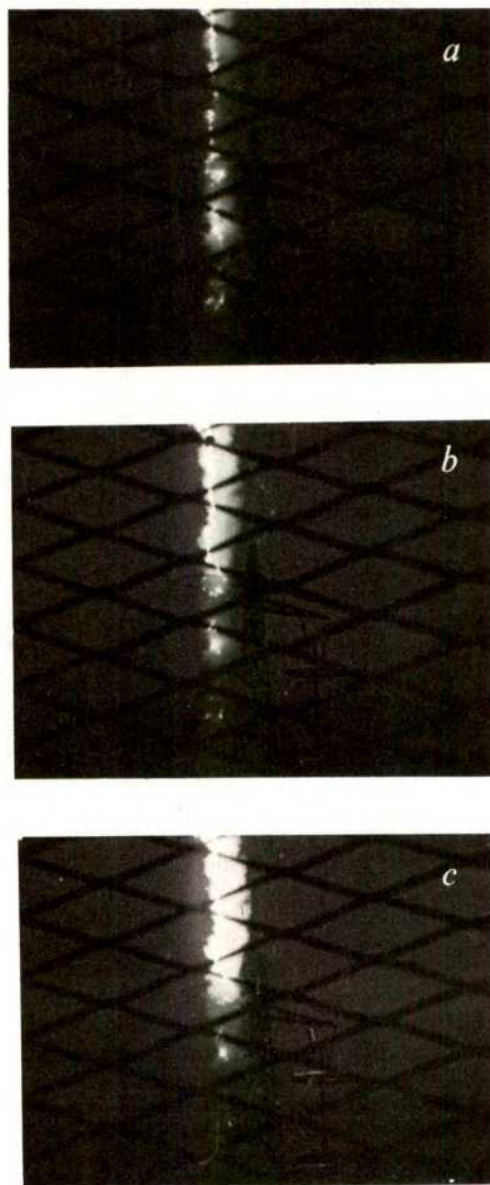
registered on pictures taken mostly with a camera (16-mm, 150 frames s^{-1}) at a distance ranging from 70 to 110 m, and with focal lengths from 30 mm to 120 mm.

The beads generally have an initial diameter of the order of 40 cm which decreases gradually with a total lifetime of 0.3 s at most.

During long-lasting strokes, the initially straight channel adopts on a progressively more tortuous shape and the biggest beads occur where tortuosity is a maximum. Since there is a positive correlation between diameter and lifetime, it follows that at the end of the decay there are one or two luminous balls only. In general these objects have an upward motion of 1 or 2 m s^{-1} , which gives an overall picture consistent with the hypothesis of a gradually cooling spheroid of hot gas¹¹.

Moreover, it seems unjustified to invoke a persistent current to explain the long lifetime¹². This results from the following observation: on one occasion, after a stroke lasting 0.66 s on the EDF tower, a luminous spheroid was floating half way up

Fig. 2 A stroke on the tower. Pictures taken with a 16-mm cine camera at 100 frames s^{-1} . *a*, At $t = 0.66$ s after initiation of a stroke reaching the platform the "beads" in the decaying channel are irregular, as is generally the case after powerful strokes of long duration; *b*, at $t = 0.68$ s a restrike occurs, illuminating the smoke trail left by the rocket, and stops on the upper structure of the tower leaving undisturbed the luminous remains below; *c*, at $t = 0.70$ s the process continues.



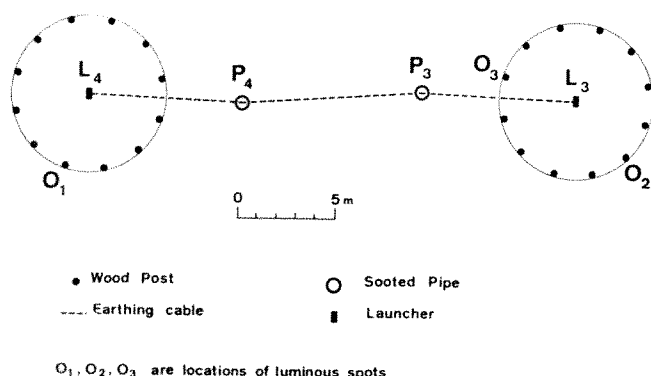


Fig. 3 Layout of a pair of launchers on the ground range and location of luminous spots.

between the launching platform and the metallic ring, then a subsequent brief stroke fell on the ring without disturbing the slow decay of the luminous object below. It is evident that, in the case of a decay governed by the input of an external current, a modification would have resulted from the change in electric field associated with the second stroke.

It can be stated also that the residue of the vaporised wire has little influence on the appearance and the behaviour of the beads. This is deduced from the following sequence of events:

In most cases the current in the first strike rises very slowly. The wire begins to break at the point where it leaves the reel, where the mechanical stress is a maximum. Then a straight arc is drawn upwards between the immobile reel and the lower end of the ascending wire. Complete vaporisation of the wire occurs only when the arc has reached a length of a few metres (typically 5 m). Then, as long as the current flows, the lower few metres emit much less light than the upper part, which is contaminated by metal vapour, but the difference disappears early during the afterglow. The similarity of the beaded decay in the lower and upper sections is indicative of the lack of influence of condensed wire residue. Incidentally, it proves also that the temperature of the beads must be rather low.

Photographic records reveal that during flashes striking pipes 3 and 4, light emission occurred at the foot of wooden posts supporting a wood fence erected for security reasons around each launcher. The general layout is shown in Fig. 3 which shows two launchers (L₃, L₄), two sooted pipes (P₃, P₄), the fences with the posts around L₃, L₄, the earthing cable (EC) and three places (O₁, O₂, O₃) where light emission has been noticed. For practical reasons the records on O₁ are more numerous and have a better quality than those concerning the two others. Since the few records on O₂ and O₃ bear much similarity with O₁ the following discussion will refer to O₁ only.

Photographic images have been obtained at a distance of 67 m with a 16-mm cine camera (150 frames s⁻¹, $f = 40$ mm) operated by us, a still camera ($f = 80$ mm) and another 16-mm cine camera (66 frames s⁻¹, $f = 10$ mm) operated by F. Rühling (Institut für Hochspannungs und Anlagentechnik, Munich). Light from O₁ has been observed twice: first during a flash on P₄ with a maximum negative current of 2.8 kA and 12 min later during a flash on P₃ with a maximum negative current of 15.3 kA. The flash on P₄ lasted 0.55 s with fluctuations but without a sharp restrike. In that case, the light from O₁ appeared at $t = 0.23$ s after breakdown, grew progressively for 0.06 s, fluctuated in phase with the flash and disappeared at $t = 0.55$ s when the main channel began to break into beads. The flash on P₃ had a total duration of 0.8 s. It began with a discharge lasting 0.42 s and ended with eight restrikes. Light from O₁ appeared at $t = 0.21$ s after breakdown, ceased at $t = 0.44$ s and appeared again with each restrike until $t = 0.8$ s.

The light-emitting region was stationary, in contact with the ground at the place where the post enters the earth. Its shape seems roughly spherical with a diameter of about 25 cm but

because of the poor resolution these observations are somewhat tenuous.

The hypothesis of an accidental reflection of light from the flash is unfounded because in both cases the object appears later than the time of maximum brightness for the main channel. Visual inspection of the spot did not reveal anything special. No marks or burns were visible on the grass or on the post.

The most probable explanation is that it was due to the influence of electric currents circulating in the ground during the flash. These currents start from the earthing cable connecting L₃, P₃, P₄, L₄, which is buried only a few centimetres deep. It is not surprising that some underground outgassing occurs and that the gases escape at the point where the posts puncture the upper layer. It remains to be decided whether the light comes from hot gases only or from a combustion involving hydrogen or methane, for instance, or from a localised electric discharge mechanism. The fact that O₁ was pulsating in synchrony with the restrikes does not support the combustion hypothesis. Further experiments are planned to clarify the question.

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Isoelectric point of the edge surface of kaolinite

THE nature of the particle-particle interactions in clay suspensions is of great technological importance since these interactions determine the flow properties of suspensions used in, for example, ceramic manufacture and paper making, and as oil drilling fluids. They also influence profoundly the structure and behaviour of soils. Clay particles are of a plate-like morphology and thus exhibit two types of surface, the edges and the basal faces. Consequently, a variety of different types of particle-particle interaction are possible in aqueous clay suspensions^{1,2}. For example, structures consisting of individual particles coagulated in an edge-face or an edge-edge arrangement have been proposed as well as face-face (or 'card-pack') aggregates which may themselves be coagulated in edge-face or edge-edge modes³.

The type of structure present in a clay suspension is determined by the electrical double layers at the two interfaces. The results presented below are a preliminary report of a study designed to evaluate the influence of electrical double layers on the properties of aqueous kaolinite suspensions³ and hence to elucidate the conditions in which the

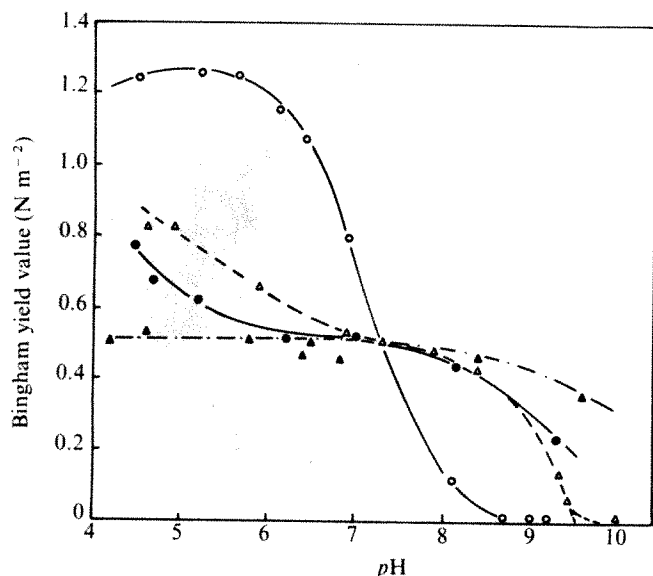


Fig. 1 Bingham yield values of homoionic sodium kaolinite as a function of pH and NaCl concentration. ○, 10^{-4} M NaCl; △, 0.017 M NaCl; ●, 0.034 M NaCl; ▲, 0.12 M NaCl.

different types of particle arrangement exist. They have also enabled a value to be determined for the isoelectric point of the edge surface of kaolinite.

The electrical double layers at edges and faces must differ either in the magnitude or in the sign of the surface potential in order to account for edge-edge and edge-face structures, since a face-face coagulated structure would be the condition of lowest free energy if the electrical double layers on all surfaces were identical and there was no potential energy barrier to coagulation. It is usually considered⁴ that the basal face of a kaolinite crystal is a surface of constant charge density, determined by the amount of isomorphous replacement within the crystal lattice; on the other hand, the edge surface has a surface potential which depends, as in the case of an oxidic surface, on the adsorption of the potential-determining hydrogen and hydroxyl ions². Schofield and Samson⁵ have demonstrated that at low pH values (below the isoelectric point of the edge surface) edge-face structures exist because the positively charged edges are electrostatically attracted to the negatively charged basal faces. At pH values slightly above the isoelectric point of the edge surface, however, the system becomes deflocculated at low ionic strengths, since the edge surface potential is negative and large, and results in high potential energy barriers to all types of coagulation.

The flow curves of clay suspensions frequently approximate to the Bingham model in which a certain yield value must be exceeded before flow is induced. The Bingham yield value is a function of the number and strength of the particle-particle linkages and is therefore a sensitive measure of the degree of coagulation. The addition of indifferent electrolyte reduces the Bingham yield value of edge-face coagulated systems by compressing the two electrical double layers and thereby reducing the electrostatic attraction. Conversely, the addition of electrolyte at high pH values increases the Bingham yield value by compressing all electrical double layers, thus lowering the energy barrier to van der Waals' coagulation. It can be argued that, at the isoelectric point of the edge surface, the system should already be fully coagulated in an edge-edge structure, even at low ionic strengths. Thus, the addition of indifferent electrolyte should have no effect on the yield value until sufficient electrolyte is added to promote face-face aggregation. If this argument is correct, then,

providing the ionic strength is not too high, plots of the Bingham yield value of kaolinite as a function of pH, each at a different ionic strength, should all coincide at one single pH value, the isoelectric point of the edge surface of a kaolinite crystal. This prediction has been validated by our study.

The rheological properties of suspensions containing 9 wt% of a highly crystalline kaolinite sample from a Cornish deposit, previously purified and converted to the homoionic sodium form according to the method of Schofield and Samson⁵, were investigated as a function of pH at different concentrations of NaCl (ref. 3). Flow curves were determined using a concentric-cylinder viscometer and in each case the data, determined at relatively high rates of shear, were extrapolated to give the Bingham yield value. Figure 1 shows the results, which are as predicted. The point of coincidence is at a pH value of 7.3 and we suggest that this is the isoelectric point of the edge surface of a kaolinite sample prepared by this method. It is clear that at pH values below this isoelectric point the yield value increases due to the onset of edge-face coagulation, whereas above it the system becomes progressively deflocculated. At pH values both above and below the isoelectric point of the edge surface, the effect of indifferent electrolyte is to change the yield value towards the value shown at the isoelectric point, that is, to promote edge-edge coagulation. This has been further corroborated³ by the fact that progressively higher electrolyte concentrations are required to achieve this yield value as the potential of the edge surface increases both above and below the isoelectric point.

The value of the isoelectric point obtained is comparable with the values obtained by others^{5,6}, and with the pH value of zero adsorption of hydrogen ions found by Flegmann *et al.*⁷. Flegmann *et al.*, however, suggested the isoelectric point of the edge surface of kaolinite to be at a pH value of 6, the maximum in the yield value-pH plot. This maximum was attributed to an edge-edge structure, but it can be seen from this work that this is a misinterpretation of the rheological data. Quirk⁸, however, has detected positive adsorption of chloride ions at pH values as high as 9-11 but it is not certain that a small amount of iron oxide impurity in his clay did not affect these results to some extent. Other values reported in the literature do not necessarily refer to clays prepared in the same way as reported here. As part of this study, certain commercial and natural clays have been investigated and the results compared with the model system described here. Different apparent isoelectric points were obtained which depended on the previous history of the clay samples. These results will be published elsewhere, along with a more detailed account of the influence of the electrical double layers on the rheological and sedimentation behaviour of the kaolinite sample reported here.

These results suggest that edge-face structures exist only below the isoelectric point of the kaolinite edge surface at low ionic strengths. Edge-edge structures exist to varying extents at all pH values at moderate concentrations (≈ 0.1 M in a 1:1 electrolyte), and at pH values around and slightly above the isoelectric point of the edge surface at low electrolyte concentrations. At higher electrolyte concentrations (> 0.1 M) it is suggested³ that face-face aggregates are formed at all pH values.

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Mechanism for shear delamination under dynamic loading

WE describe here a debonding mechanism for interfaces between dissimilar materials in conditions of dynamic loading, and we deal, in particular, with coated substrates that are impacted by solid or liquid projectiles. Protective coatings are commonly used to enhance the resistance of materials to impact erosion; for example, non-metallic fibre-reinforced composite materials are found to be very susceptible to impact erosion unless a suitable thin coating is applied. For these composites, certain elastomers can be successful as coating materials, even though they do not, intrinsically, possess high strength. Coatings of this type reduce the pressures that are transmitted to the substrate by presenting a lower dynamic impedance to the impacting body. The interfacial adhesion between the coating and substrate is of great importance because it enables the substrate to reinforce the coating by restricting the lateral strain, and therefore the stress, within the coating. When adhesion is lost, the coating itself becomes quickly eroded. Thus, the bond between the coating and substrate is often of great significance to the success of a coating-substrate system.

The impact of liquid drops on to a coated substrate has been considered by a number of authors^{1–3}, but little attention has been paid to the precise mechanisms of the adhesion breakdown between the coating and substrate. High speed photographic observations of the process of delamination under liquid impact^{4,5} and examination of

Fig. 1 Experimental arrangement for the high speed photography.

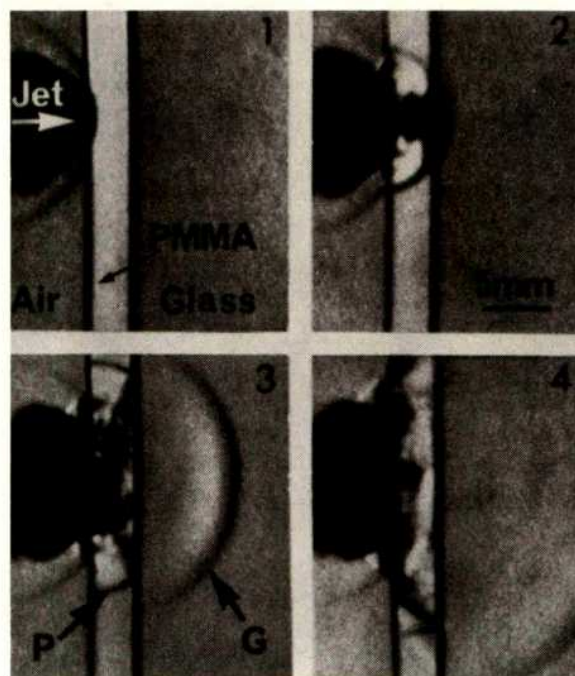
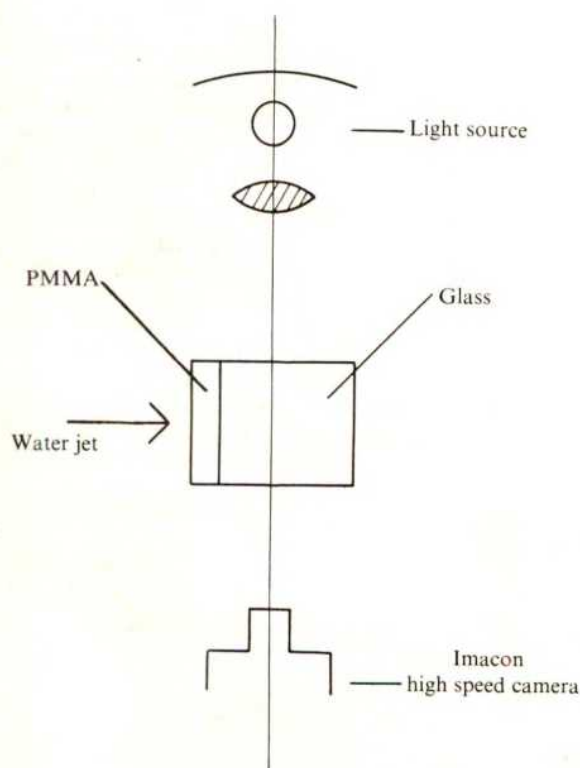


Fig. 2 The front of a water jet (750 m s^{-1}) impacting a PMMA layer that is bonded to a glass block. The jet head diameter is $\approx 3 \text{ mm}$. Frames are selected from an Imacon sequence of $10^6 \text{ frames s}^{-1}$. P, Compressive stress front in the PMMA; G, transmitted stress in the glass block.

impacted laminates have shown that the delaminations are usually composed of distinct coaxial annular regions. This suggests that several mechanisms are operative. Often the interface on the impact axis has not failed, and the total delamination is an annulus. This behaviour cannot be explained by the commonly cited mechanism of tensile failure resulting from reflected or dispersed stress waves, because such interactions lead to maximum tensions developing on the impact axis. Therefore the creation of shear stress along the interface must be considered. When stress waves from an impact meet an interface, shear stresses can be set up by several mechanisms; for example, they can arise from the incident pulse of shear stress (which is usually of a low magnitude) or from the oblique incidence of the main compressive pulse⁶. The high speed photographic sequences presented here illustrate another form of shear debonding of comparable magnitude to the latter. The mechanism arises from the wavefront separation that occurs when stress propagates parallel to an interface between two media which have different stress-wave velocities.

A schematic diagram of the experiment is shown in Fig. 1. The front of a water cylinder impacts a polymethylmethacrylate (PMMA) layer 3 mm thick, that is bonded to a glass block. The jet velocity in the experiments was 750 m s^{-1} (see also refs 7 and 8). A sensitive shadowgraph optical system⁴ was used to make the stress waves generated by the impact visible. The high speed photographs were taken with a Hadland Imacon framing image converter camera operating at $10^6 \text{ frames s}^{-1}$, and selected frames from two sequences are shown in Figs 2 and 3.

When the front of the jet first strikes the PMMA surface, the liquid behaves compressibly for about $1 \mu\text{s}$ and a very intense pressure, $\sim 1 \text{ GPa}$ is developed⁷. When the pressure front in the PMMA, P, reaches the PMMA-glass interface, it generates the transmitted pulse G in the glass. The stress propagation velocity in the glass is high ($5,600 \text{ m s}^{-1}$) compared with that in the PMMA ($2,700 \text{ m s}^{-1}$ to $\sim 3,200 \text{ m s}^{-1}$, depending on the stress level). Therefore, there is a critical

angle of incidence of the diverging pulse P beyond which no stress is transmitted across the interface. A non-delaminated region corresponding to the size predicted by this angle is found during high speed photographic observation of coated substrates⁵. Outside this diameter in the region of the interface, front G in the glass overtakes pulse P travelling in the PMMA: these separated fronts are seen clearly in frame 3 of Fig. 2. In frame 2 of Fig. 3 disturbance H, a 'headwave' in the PMMA, is generated by the faster stress front, G, in the glass. It indicates that a high stress is being transmitted across the interface by the separation of the wave fronts, that is, that shear strain is developed between stressed glass and unstressed PMMA. This is a potential delamination mechanism.

The theory of stress waves in these conditions has been developed by many authors (see, for example, refs 9 and 10). The conditions for delamination and its effect on the subsequent wave propagation are not, however, discussed. High speed photographic studies of spherical stress waves^{11,12} have shown that both the normal and tangential stresses at the interface achieve large values at various stages in the propagation history. The analogous situation of plane wavefronts propagating parallel to the boundary between coupled bars of dissimilar media has also been considered by a number of authors (see, for example, refs 13 and 14). They have shown that the coupling force can be of sufficient magnitude to cause debonding.

Observations of the formation of delamination fractures have shown that it is a very complex process, and involves many different mechanisms. Velocities of delamination growth exceed the maximum tensile crack velocities, and vary according to the stress wave propagation conditions. The simple mechanism illustrated here may not be the only one operating, but it does involve stress levels that are significant for many bonded interfaces. Even if it does not cause complete failure of the bond, it may cause nucleation of local failure sites which are then extended by the succeeding stress history.

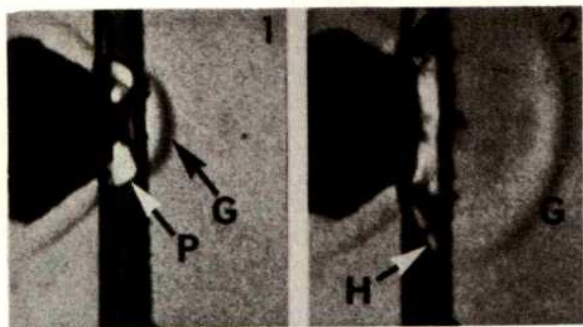


Fig. 3 As Fig. 2, but different illumination conditions also show the headwave, H. 1 μ s between frames.

An important point to note is that this mechanism will operate between media which are 'acoustically matched' (that is, media which have the same value for the product ρC , where ρ is the density and C is the stress wave velocity). Acoustic matching has often been considered as a means to minimise interfacial stress by eliminating reflected wave components. The shear mechanism of wavefront separation, which relies on a mismatch of C and not ρC , will, however, still in general operate. We have observed annular delaminations between such acoustically matched layers, which can be explained by this process. We conclude that, although acoustic matching of coatings may be beneficial, it is also important to choose materials which have similar values of C . In practice, this may be difficult to achieve by using a single coating material, but multiple coatings, with

a gradation of physical properties, could overcome this problem.

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Large scale changes in North Sea phytoplankton

CONTINUOUS plankton recorders (CPRs) are towed^{1,2} at approximately monthly intervals in the North Sea along standardised routes at a standard depth of 10 m. Analysis of long term results has revealed large scale trends and changes³. Here I deal with changes in the phytoplankton from 1948 to 1973 as evidenced by results from area C2 (Fig. 1) in the western North Sea.

As the CPR is towed through the water it filters the plankton on to a continuously moving band of silk with apertures of approximately 270 μ m. Phytoplankton is analysed in these samples in two ways. First, 'phytoplankton colour' is visually assessed into three categories⁴ to give a relative estimate of

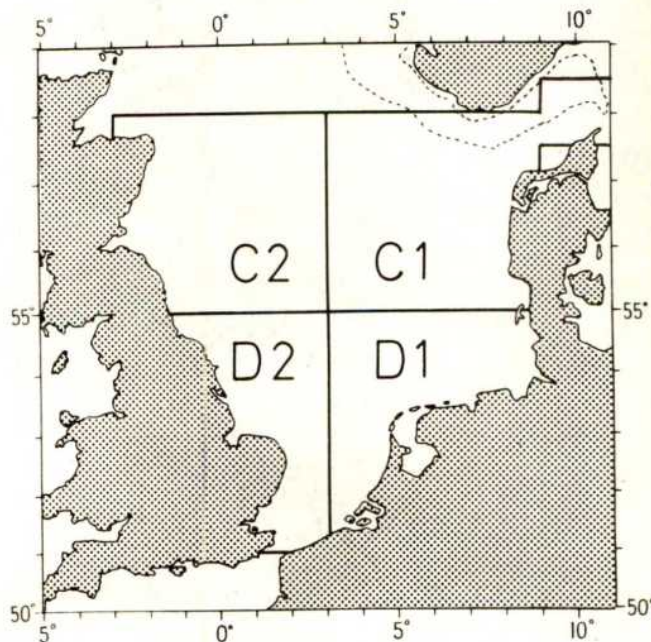


Fig. 1 North Sea standard areas.

Table 1 Species included in diatom and *Ceratium* spp. totals

Diatoms	<i>Ceratium</i>
* <i>Paralia sulcata</i> (Ehrenb.) Cleve	<i>Ceratium fusus</i> (Ehrenb.) Dujardin
* <i>Skeletonema costatum</i> (Grev.) Cleve	<i>Ceratium furca</i> (Ehrenb.) Clap. & Lachm.
* <i>Thalassiosira</i> spp.	<i>Ceratium lineatum</i> (Ehrenb.) Cleve
* <i>Dactyliosolen mediterraneus</i> Perag.	<i>Ceratium tripos</i> (Müller) Nitzsch
* <i>Rhizosolenia imbricata</i> forma <i>shrubsolei</i> (Cleve) Schröder	<i>Ceratium macroceros</i> (Ehrenb.) Vanhöffen
* <i>Rhizosolenia styliformis</i> Brightw.	<i>Ceratium horridum</i> (Cleve) Gran
* <i>Rhizosolenia hebetata</i> forma <i>semispina</i> (Hensen) Gran	<i>Ceratium longipes</i> (Bailey) Gran
* <i>Rhizosolenia alata</i> forma <i>indica</i> (Perag.) Gran	
* <i>Rhizosolenia alata</i> forma <i>alata</i> Brightw.	
* <i>Hyalochaeta</i> spp.	
* <i>Phaeoceros</i> spp.	
* <i>Asterionella glacialis</i> Castr.	
† <i>Thalassiothrix longissima</i> Cleve & Gran	
* <i>Thalassionema nitzschioides</i> Hust.	
* <i>Nitzschia seriata</i> Cleve	
* <i>Nitzschia delicatissima</i> Cleve	

*All species typically form chains which increases their chance of being entrapped in the CPR silk.

†The large species *T. longissima* occurs free but frequently forms dense matted colonies.

All *Ceratium* species are large and likely to be well sampled by the CPR.

phytoplankton standing crop (visual chlorophyll). Second, since 1958, species have been identified and counted⁵. Most phytoplankton passes through the coarse meshes of the CPR but comparisons with water samples⁶ and the results of the survey indicate that the portion retained seems to reflect changes in the abundance and distribution of the major components of the phytoplankton. The large number of CPR samples taken over extensive areas smooths the effect of patchiness and gives gross

estimates of abundance which are comparable within the CPR survey.

The monthly means for both methods of analysis are calculated for samples in 2° longitude × 1° latitude rectangles and then averaged into larger 'standard areas'. Results for Standard Area C2 are presented as contoured month/year diagrams in Figs 2–4. The pattern of phytoplankton change observed in Standard Area C2 is, in many respects, typical of the whole of the North Sea. *Ceratium* spp. and diatoms represent the totals of the average abundance of the seven most common *Ceratium* species and 16 diatoms in the North Sea (Table 1).

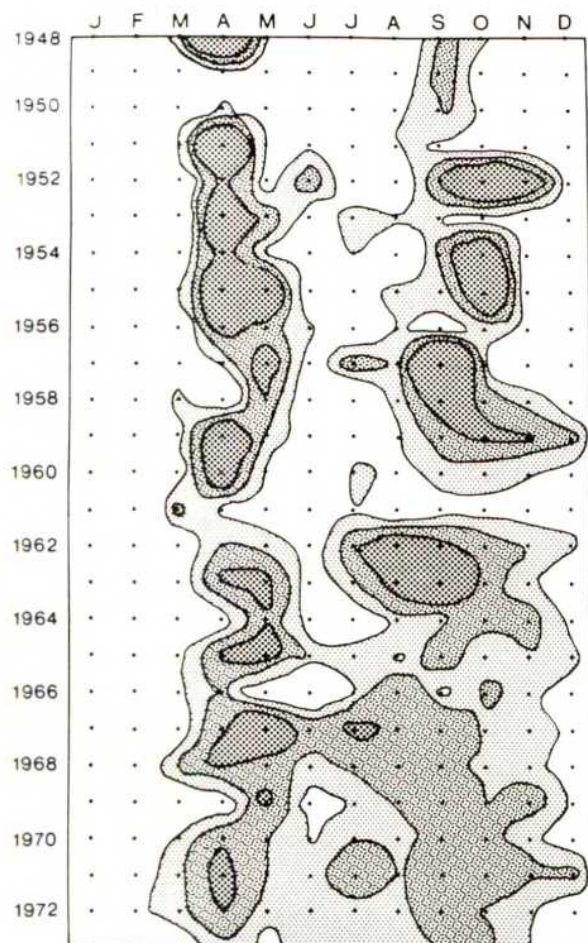
Before 1965, the analyses of phytoplankton colour revealed distinct spring and autumn blooms (Fig. 2) but after that date the distinction between the two blooms became less marked and the productive season became longer, although the autumn bloom declined in intensity. *Ceratium* spp. (Fig. 3) have shown little change in either timing or abundance since 1958.

The diatom count decreased markedly after 1965 (Fig. 4). The autumn bloom declined drastically and winter and summer diatoms almost disappeared. A more gradual but still considerable decline occurred in the spring bloom.

To some extent, there seems to be an inverse relationship between the pattern of phytoplankton colour and diatom changes. The diatoms have declined in abundance (especially in the summer, autumn and winter months) as phytoplankton colour has increased. Therefore, it seems that phytoplankton other than diatoms and *Ceratium* spp. as measured by the CPR has caused the general increase in colour. Other groups such as the silicoflagellates and coccolithophores only occurred in small numbers and *Phaeocystis* showed a decline similar to that of the diatoms after 1965. One possible explanation is that there may have been an increase in the abundance of microflagellates which partially disintegrate in formalin leaving their chloroplasts to add to the coloration of the silks. Such microflagellates would not be identifiable in CPR samples but could have caused the increase in phytoplankton colour. A similar replacement of a diatom by a flagellate population was described by Grøntved⁷ as part of the seasonal succession of the phytoplankton of the North Sea during 1947.

The pronounced change in the phytoplankton of area C2 which took place around 1965 is coincident with the return of plankton, which was typical of the 1920s, to the English Channel off Plymouth⁸. This reversal in Channel plankton was attributed by Russell *et al.*⁸ to climatic change which may have led to an increased Atlantic inflow into the North Sea. Unfortunately, the CPR survey did not commence in the North Sea until 1931 so that it is not possible to determine whether the recent

Fig. 2 Phytoplankton colour (visual chlorophyll) in Standard Area C2.



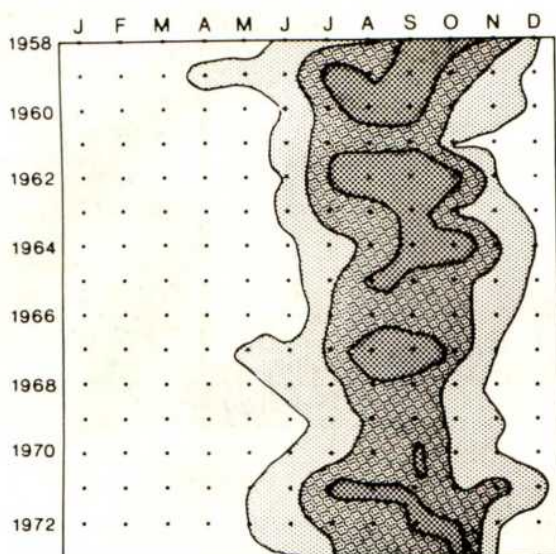


Fig. 3 Abundance of *Ceratium* in Standard Area C2.

changes in the North Sea are a return to the conditions of the 1920s.

If climatic changes are causing the considerable changes in the plankton of the North Sea and the English Channel, then they seem to be influencing the zooplankton and phytoplankton of the two areas in different ways; both zooplankton biomass and diatoms are increasing in the English Channel off Plymouth⁸, whereas in the North Sea they are declining⁹. But productivity as measured by ¹⁴C has increased in the English Channel since 1965 (ref. 8). While there are no comparable time series of productivity measurements in the North Sea, the standing crop as determined from crude estimates of phytoplankton colour certainly seems to have increased.

A second possible alternative cause of the phytoplankton changes is eutrophication caused by increasing nutrient levels⁹ in the North Sea. This seems unlikely, however, because eutrophication arising from pollution, only seems to be a potential problem in coastal waters¹⁰ and not in the offshore waters of area C2.

The changing composition of the phytoplankton in area C2 since 1965 is likely to be reflected at higher trophic levels. During the same period there has been a parallel decline in the

zooplankton biomass of the North Sea³. It is possible, therefore, that the plants which seem to be replacing the diatoms since 1965 may be less readily utilised as food. Contemporary changes have also taken place in the fish larvae of the CPR survey (S. H. Coombs, personal communication). We are now attempting to identify the organisms which seem to be replacing the diatoms and to determine whether the changes in the North Sea are cyclical and related to climatic change or to other as yet unknown influences.

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Is the illusory triangle physical or imaginary?

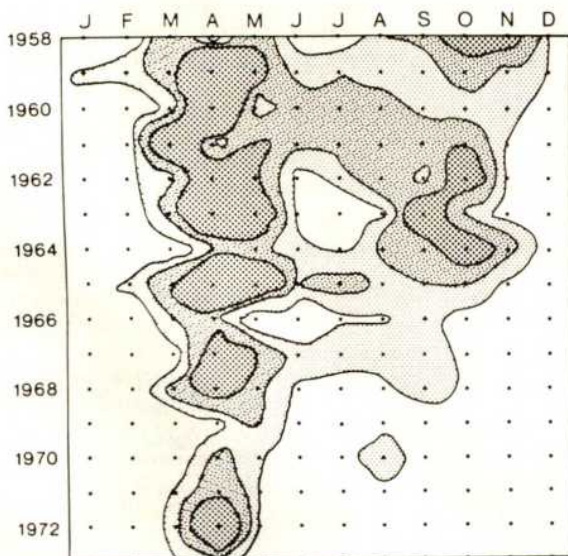
MOST pictures of visual interest contain a large amount of detailed information. Studies have shown that a great deal of these data can be filtered out without destroying a subject's or a machine's ability to recognise even quite complex objects¹⁻⁴. The resulting benefits are economy of memory store and considerable flexibility in the shape and detail of objects classified as the same.

Such filtering must to some extent distort our own visual perception and can account for certain geometric and contrast illusions⁴. There has been recent interest in illusory contours⁵. Figure 1a shows an example of an illusory contour perceived in a Kanizsa triangle. A black triangle is perceived as a unitary form separated in depth over a white triangle. The question now arises, what would happen if we filter this figure to get rid of the redundant information? In other words, what form remains if the detailed information (the higher spatial frequencies) not necessary for the identification of the original pattern features are removed?

This filtering was carried out as follows. The two-dimensional discrete Fourier transform of the original picture was made (Fig. 1b). (A log transform was used on both the transform and filtered pictures to compress the intensity levels and provide the maximum detail.) The magnitudes of these spatial frequencies were decreased according to the attenuation characteristics of the visual system⁶ (Fig. 1c). An inverse Fourier transform reconstructs this attenuated spatial frequency data (Fig. 1d). The filtered picture results in smoothed pattern features and a loss in contrast in the lines, but enough resolution is still retained to provide an illusory triangle. There is still no clue from this picture, however, as to how the original pattern features interact to provide the illusory triangle.

Further spatial filtering was carried out to remove even more redundant pattern information. Previous experiments on filtering suggest that the low spatial frequencies will provide sufficient information for classifying forms¹⁻⁴. When this filtering was accomplished by removing three-fourths of the higher spatial frequencies (Fig. 1e), we note a generally homogeneous dark triangle surrounded by a somewhat homogeneous lighter

Fig. 4 Abundance of diatoms in Standard Area C2.



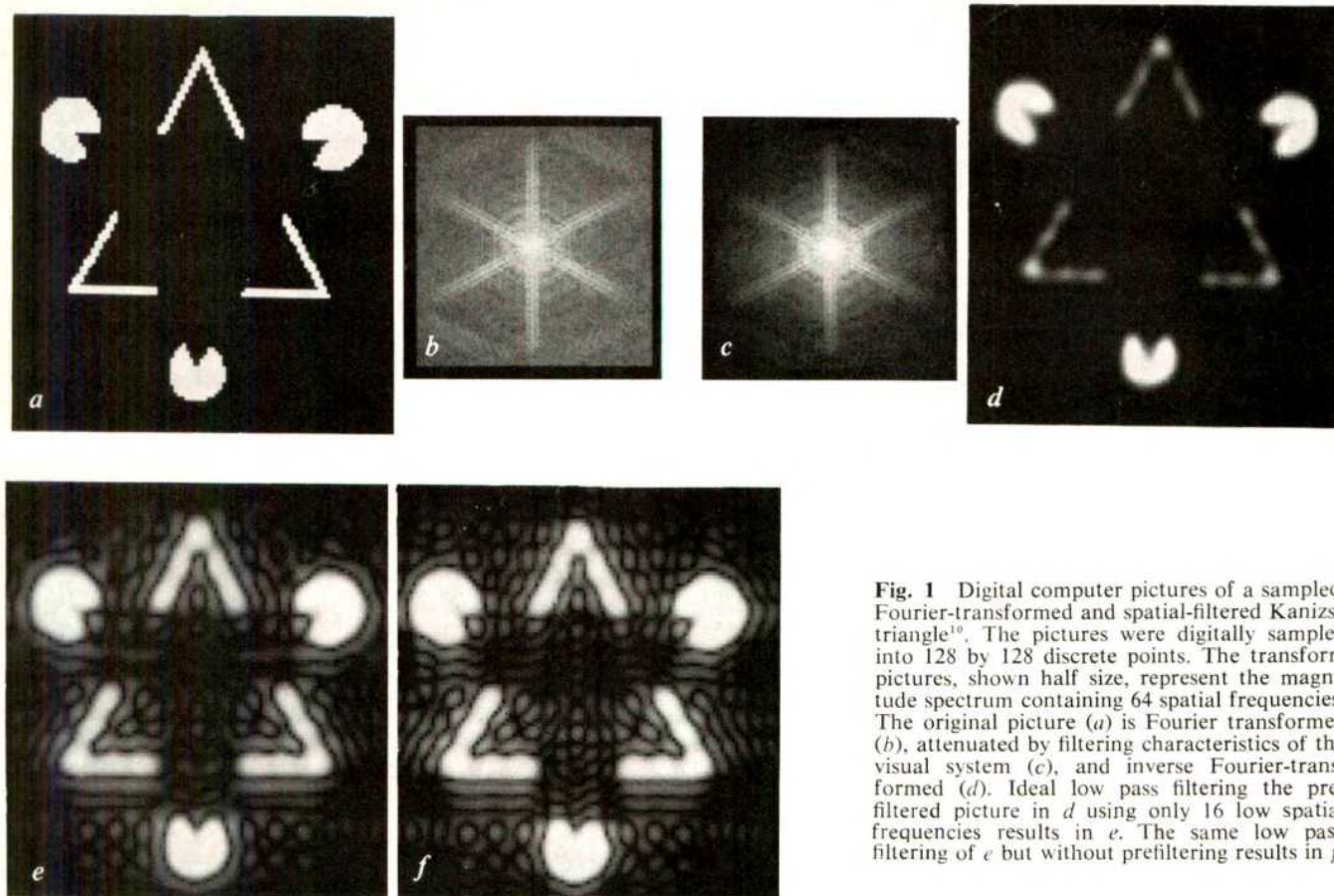


Fig. 1 Digital computer pictures of a sampled, Fourier-transformed and spatial-filtered Kanizsa triangle¹⁰. The pictures were digitally sampled into 128 by 128 discrete points. The transform pictures, shown half size, represent the magnitude spectrum containing 64 spatial frequencies. The original picture (a) is Fourier transformed (b), attenuated by filtering characteristics of the visual system (c), and inverse Fourier-transformed (d). Ideal low pass filtering the pre-filtered picture in d using only 16 low spatial frequencies results in e. The same low pass filtering of e but without prefiltering results in f.

triangle and area. Note in particular the top dark horizontal boundary line of the dark triangle. (This sharp boundary results from the most linear sectorised disk and smooth line features of the original pattern. Other similar pattern features of the original triangle are jagged because of the coarse quantification process, and result in less well defined regions in the filtered pictures. Further improvements in techniques will provide even more striking results.) It seems that as in previous results, limited regions of low spatial frequencies do provide pattern information that is not readily apparent from analysing the original pattern features.

Have the initial filtering characteristics of the visual system attenuated the low spatial frequency information in such a manner as to aid the detection of the 'illusory' triangle? We answer this question by low pass filtering the original picture directly. The resulting picture (Fig. 1f) shows a somewhat less homogeneous dark triangle region. This result suggests that the low spatial frequency attenuation characteristics of the visual system do aid the formation of the 'illusory' triangle.

To convince a blind mathematician that a triangle really exists, one could have simply correlated the original pattern with an actual triangle. Correlation would provide an independent and objective measure of the degree to which the illusory triangle exists in the original pattern. Previous pattern classification studies, however, have shown that the low spatial frequencies provide generalised form information that is relatively insensitive to discriminable differences between objects classified as the same. Therefore, to be consistent with previous analysis and not treat this pattern in any way different from others, we compared just the low spatial frequencies of the original pattern with the low spatial frequencies of a black triangle of similar size and orientation. They correlate.

It should be stressed, however, that not all subjective contour patterns may be explained so easily by such filtering or correlation techniques. If the sectorised disks are replaced with small dots, as Gregory has done, an illusory triangle does exist although it is markedly less evident⁵. Initial filtering analysis of a similar pattern has yielded promising but not too convincing

results, because of the relatively small energy contained in the dots. Again, further research will be needed to improve techniques and determine whether or not additional mechanisms are being used to detect and process other illusory contours.

In summary, the Kanizsa triangle has been filtered in the above well defined manner. The final picture contains a black triangle, but mathematically we have not informed the system used about a triangle. We have not explicitly manipulated the original pattern features, nor have we provided additional cognitive clues. Neither the Fourier transform nor the filtering acting on the original pattern have generated the illusory contour triangle. Rather, the filtering has isolated and enhanced the illusory triangle information that was implicit in the overall spatial relationships of the original pattern features. Therefore the original picture must have contained the triangle. It is therefore not surprising that our visual system detects it. This analysis is consistent with visual spatial information being filtered by quasi-independent spatial frequency-orientation channels as evidenced by neurophysiological and psychophysical studies⁷⁻⁹.

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Intrauterine nutrition and aggression

WITH the increase in conflict and aggression in human society, the underlying causes of aggression take on added significance. The laboratory investigation of human aggression is difficult primarily because of ethical considerations. Animal aggression must therefore serve as a model for the experimental study of suspected causes of aggression. One potential cause is malnutrition, which has been assessed by studies of the effects of prenatal zinc deficiency and intrauterine undernutrition on aggressive behaviour in the adult rat. Prenatal zinc deficiency was chosen because it has been found to significantly reduce the size of the brains of foetal¹ and neonatal rats², to impair the synthesis of DNA in the rat brain³, and to decrease the activity of RNA polymerase in neonatal rat liver⁴ and brain⁵. When zinc deficiency is very severe an increased incidence of central nervous system teratology occurs in the foetal rat⁶. Behavioural deficits such as impaired avoidance learning have also been observed^{1,7}.

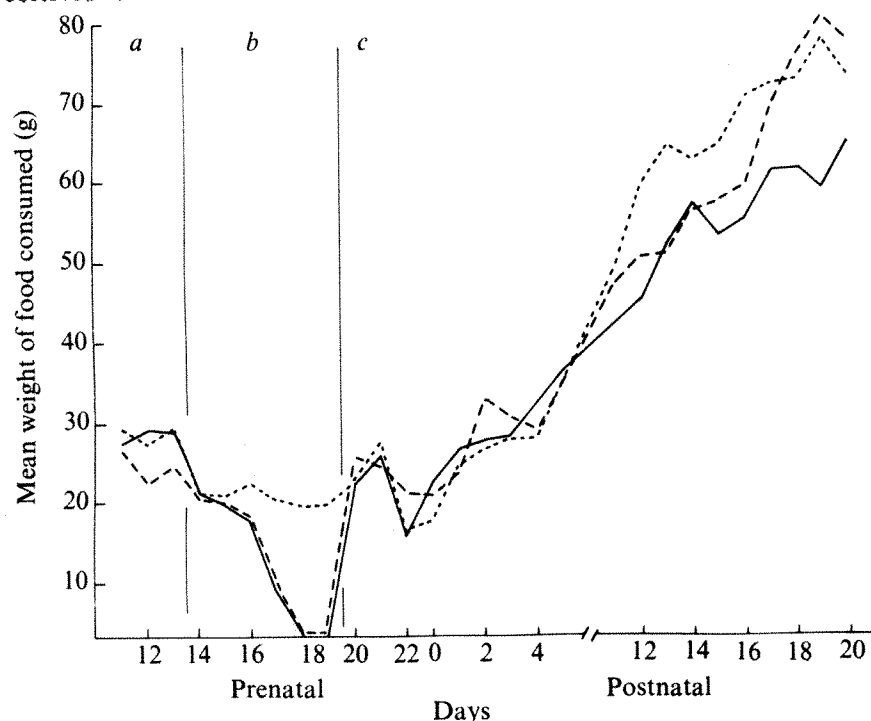


Fig. 1 Comparison of food consumption among three groups of dams. AL dams (.....) ate significantly more food ($P < 0.001$) than the ZD dams (—) during the zinc-deficient period. PF dams (---) were given the same quantity of food as eaten by the ZD dams during this period. There were no differences among the three groups during the normal diet periods. Day 0 is the day of delivery. a, Normal diet; b, zinc-deficient diet; c, normal diet.

This report shows that adult female rats whose dams suffered prenatal zinc deficiency (ZD) or undernutrition as a result of pair feeding (PF) are significantly more aggressive at a high level of shock (1.6 mA) than adult female rats whose dams were fed *ad libitum* (AL) throughout pregnancy; and that the ZD female rats are more aggressive than the PF female rats. At a lower level of shock (1.3 mA), PF and AL female rats display a similar level of aggression well below that of the ZD female rats.

Thirty pregnant Long-Evans rats were randomly divided into three groups. The dams were housed in plastic cages in a room that was controlled for temperature and humidity. Starting on day 14 of pregnancy, 10 dams (ZD) were fed a biotin-enriched sprayed egg-white diet which contained less than $1 \mu\text{g}$ zinc per g (ref. 2) and deionised water. As zinc deficiency causes anorexia, a second group of 10 dams was individually paired (PF) with the ZD dams and fed the same quantity of the diet as was consumed by their ZD partners (Fig. 1). The PF dams were supplemented with $50 \mu\text{g}$ zinc per ml of drinking water. A third group of 10 dams (AL) were fed the same diet *ad libitum* and given the zinc-supplemented water. On day 20 of pregnancy, all dams were taken off the zinc-deficient diet and fed Purina labora-

tory chow⁸ *ad libitum* and tap water. After delivery, all the dams and their pups were continued on the chow *ad libitum* and tapwater. The reduced food consumption during the zinc-deficient period had a marked effect on the mean weights of the ZD and PF dams (Fig. 2). For the first 40 d of life, the ZD pups were smaller than the PF and AL pups, but by day 75, there were no differences among the three groups (Table 1).

When the pups were 75 d old, 10 females from each group were randomly selected and tested for aggression. The 10 females in each group were divided into five pairs for a total of 15 pairs. Each pair was tested for aggressive behaviour in a clear Plexiglas chamber ($32.0 \times 25.5 \times 30.5$ cm). The grid floor consisted of 0.3-cm stainless steel rods spaced 1.9 cm from centre to centre. The rods were electrified by a Grason-Stadler Model 700 shock generator. The chamber was housed in a box 80 cm high, 61 cm deep and 80 cm wide. Two fans provided intake and exhaust ventilation. Each pair of rats was given 25 shocks per d for 10 d. A modified counter balance procedure (ABBA) was used. On the first 2 d of shock, the intensity was 1.6 mA followed

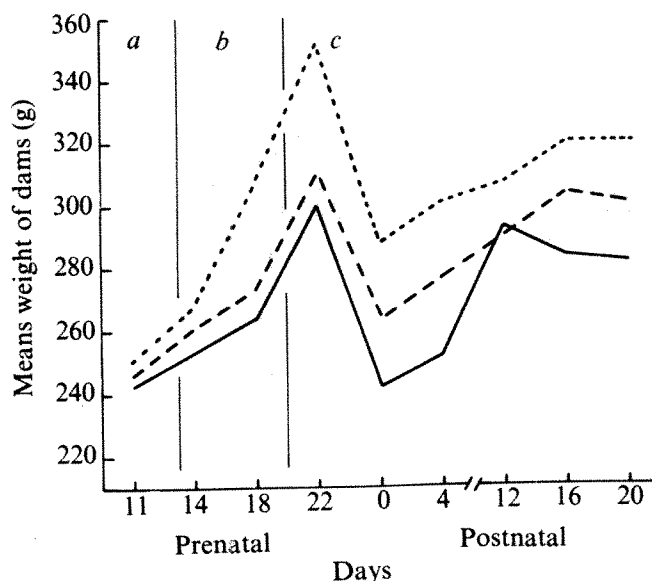


Fig. 2 Mean weight of dams during the prenatal and postnatal period as a function of dietary manipulations during days 14–20 of pregnancy. See Fig. 1 for coding.

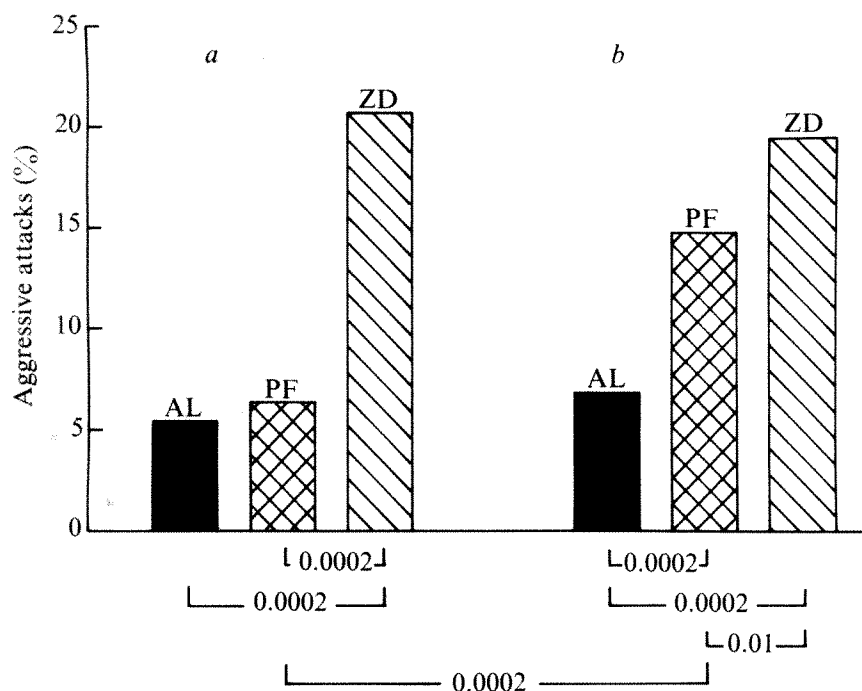


Fig. 3 Comparison of the level of aggression among the three groups of rats at two different levels of shock. ZD rats were significantly more aggressive than either PF or AL rats at both levels of shock. PF rats were significantly more aggressive than the AL rats at 1.6 (b) but not at 1.3 mA of shock (a).

by 5 d of shock at 1.3 mA. The shock was 1.6 mA for the final 3 d. All rats were given 1 d rest after each day of shock. All shocks were 0.5 s in duration and spaced 5.0 s apart. The sessions were videotaped and later evaluated independently by three judges who counted aggressive attacks per pair per session. Aggressive attacks were defined as 'directed movement toward the opponent' which resulted in contact, including at least one of the following responses: biting, sparring, upright attack posturing, or supine submissive posturing adopted by the attacked rat⁸. All judgments were made independently and without knowledge of the group origin of each pair. The reliability between the judges, computed by analysis of variance estimate of reliability⁹, for each day, ranged from 0.95 to 0.99 for the 10 d.

Figure 3 shows the percentage of aggressive attacks displayed by the three groups for the two levels of shock. The total percentage for each group represents the percentage of shocks resulting in aggressive attacks observed in each of the five pairs over 125 trials or 625 trials for the group. Differences between these proportions were compared for statistical significance using a grand combined proportion of aggressive attacks in the error term¹⁰. The level of significance between groups is shown in brackets below the group columns. Only those probabilities $P \leq 0.10$ are shown.

The results show that the AL rats were significantly less aggressive than the ZD rats regardless of the level of shock. Furthermore the levels of aggression remained unchanged for both groups even though the level of shock changed. The offspring of the PF rats who had suffered intrauterine undernutrition¹, were highly aggressive at a high level of shock but were no more aggressive than the AL rats at a lower level of shock. These results suggest that animals that experience prenatal undernutrition during the critical period for brain growth will be more aggressive than normal animals. The results also suggest that animals that experience zinc deficiency during the same period of intrauterine

development are more severely impaired and are even more aggressive than prenatally undernourished rats. Although extrapolation to human aggression is premature, these results do suggest the need for additional research in the field of malnutrition and aggression.

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Induction of plants from pollen grains of *Petunia* cultured *in vitro*

THE ease with which plantlets can be produced from somatic tissue, protoplasts and anther cultures of *Petunia*¹⁻³ has prompted us to use isolated pollen grain culture⁴ to determine precisely the stage at which pollen elicits the best androgenic response, with a view to increasing the yield of haploid plants thus produced.

Flower buds in various stages of development from two strains of *Petunia hybrida* L. (Cascade and Rose du Ciel) grown in the greenhouse (the phytotron at Gif-sur-Yvette) were sterilised in a filtered suspension of calcium hypochlorite (7%)

Table 1 Mean weights (g) of pups at 0, 20, 40 and 75 d of age

	0	20	40	75
ZD	5.48	29.8	107.8	224.6
PF	6.09	36.3	129.6	220.6
AL	6.11	37.5	131.2	219.3

for 5 min and rinsed three times with sterile distilled water. Fifteen to twenty anthers were homogenised in a Potter-Elvehjem homogeniser tube with 10 ml of culture medium. The suspension thus obtained was filtered through two superposed sieves of 53 and 73 mesh. New medium was added to give the final concentration desired. This inoculum contained microspores and/or pollen grains according to the age of the anthers used. A large amount of pollen could thus be obtained. The inoculum was rinsed three times with new medium after centrifugation at 120g for 5 min and resuspended in the fresh medium. The pollen grains were cultivated in plastic Petri dishes (6 cm diameter) containing 3 ml of solution inoculated with 10^4 pollen grains per ml. The dishes were maintained at 25 °C for 7 d in the dark and then illuminated continuously with 1,000 lx at a temperature of 28 ± 1 °C. The culture medium consisted of macroelements according to Murashige and Skoog (we used half the concentration described in ref. 5), the microelements and vitamins of Nitsch and Nitsch⁶ supplemented with auxin, cytokinin, boric acid (25 mg l⁻¹), sucrose (2%) and glucose (2%). The pH was adjusted in all cases to 5.6 before autoclaving.

Greenish calluses appeared gradually from the pollen 10–20 d after inoculation into the medium containing various combinations of benzyladenine (BA) and naphthalene acetic acid (NAA). As Table 1 shows, that calluses formed in the combination of BA (0.1 mg l⁻¹) and NAA (0.1 mg l⁻¹) were much more numerous than in other combinations tested. After 30 d of culture 50 pieces of pollen-callus from *P. hybrida* Rose du Ciel were transferred to the same medium; 30 of them gave rise to shoots, 12 produced compact green calluses and 8 did not differentiate.

Fig. 1 *a*, A pollen plant of *P. hybrida* L. Rose du Ciel ($n = 7$), ($\times 1$). *b*, pollen stages: *b*, binucleate; *m*, at first haploid mitosis; *u*, uninucleate ($\times 800$); *c*, and *d*, dividing pollen grains (arrows); *e*, 1 week of culture, $\times 300$; *d*, 2 weeks of culture, $\times 220$; *e*, pollen calluses after 30 d ($\times 1.5$); *f*, shoots from pollen callus after 45 d ($\times 1.5$).

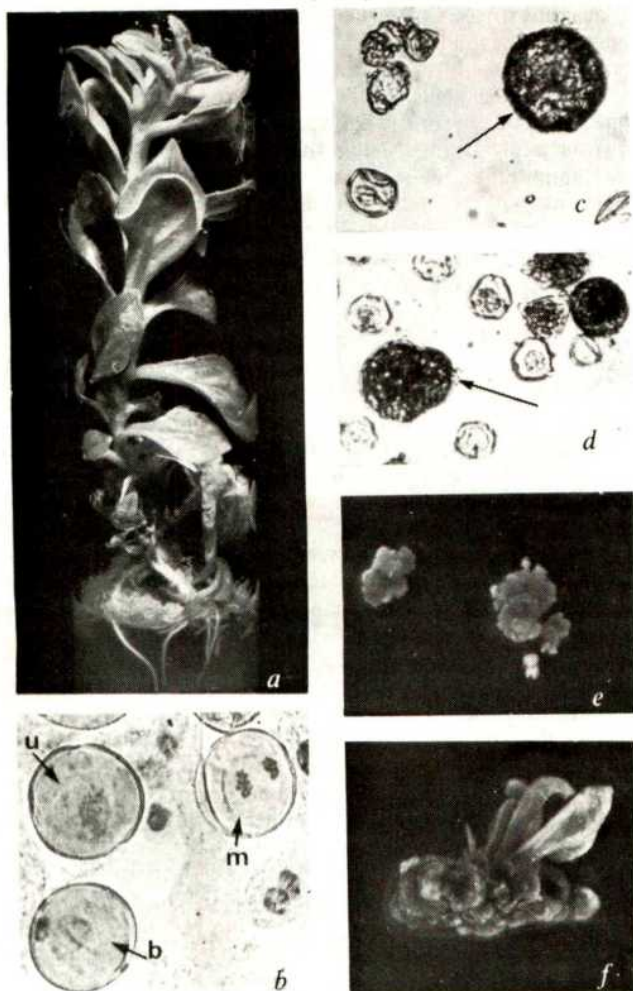


Table 1 Effect of different concentrations of NAA and benzyladenine on formation of pollen calluses in *P. hybrida* L.

Composition of the medium:		No. of calluses formed from isolated pollen grains	
NAA (mg l ⁻¹)	Benzyladenine (mg l ⁻¹)	Rose du Ciel	Cascade
0.1	0.1	2	0
0.5	0.5	13	8
0.1	1	61	58
1	0.1	32	36
1	1	25	30

Pollen extracted from 300 anthers at first pollen mitosis was inoculated for each treatment. Results were scored after 1 month of incubation.

Roots were initiated when the shoots were transferred into basal medium with indolyl-3-acetic acid (0.1 mg l⁻¹) or NAA or without growth hormones. Once they have formed an adequate root system the plantlets (Fig. 1) can be transplanted to pots and raised to mature plants. The same result was obtained when *P. hybrida* Cascade was tested for organogenesis.

The pollen stage at the time of inoculation is an important factor, determining the success or failure of pollen culture. According to our results the microspore inoculated at an early, mid or late uninucleate stage did not divide in any medium or combination tested. Pollen inoculated at, just before or just after the first mitosis (Fig. 1*b*), however, responded to the various media tested with essentially the same results for the two varieties (Table 1).

The course of callus formation in androgenic pollen of *P. hybrida* Rose du Ciel cultured *in vitro* was also followed. Some pollen grains (5–10%) enlarged considerably during the first week of culture (Fig. 1*c*) to form round multicellular masses (Fig. 1*d*), whereas others (90–95%) degenerated progressively. During the third week of culture the round multicellular masses produced irregular calluses (Fig. 1*e*). The formation of many shoot apices could be observed on the same callus within 40 d but only some of them formed shoots after prolonged incubation in the culture medium or after transfer to fresh medium (Fig. 1*f*). As these data show, only a small percentage of pollen grains *P. hybrida* developed into plants—approximately one plant developed from the pollen of eight anthers (that is 20% androgenic anthers). This, however, was a greater percentage of plant morphogenesis than Raquin and Pilet achieved with anther culture (1% androgenic anthers)⁸.

Cytological examination of the root-tip squashes of regenerated plants revealed haploid, diploid ($2n = 14$) and triploid plants among the population. The frequency of haploid plantlet formation was generally low compared with diploid or triploid; of 30 plants initiated from pollen culture, three were haploid, seven diploid and 20 triploid in *P. hybrida* Rose du Ciel. The existence of ploidy differences among the adult population is common in plantlets derived from microspores and can be accounted for by endomitosis or nuclear fusion: in such cases they will be homozygous^{7–10}. Thus, homozygous diploids obtained by pollen culture could eliminate long delays in the production of inbred lines.

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Nerves and angiogenesis in amphibian limb regeneration

In the past 150 yr, since the requirement of nerves for amphibian limb regeneration was demonstrated, there has been considerable progress in specifying the nature of the phenomenon but virtually none in understanding the site or nature of the neuronal effect¹. If denervation is carried out early, amputated limbs will not regenerate and may even undergo regression; at later stages in regeneration, when a blastema has formed, regeneration is not prevented by denervation, although in the newt at least, growth in volume is inhibited while growth in length and morphogenesis continue^{2,3}. Studies on the neuronal dependence have shown that any nerve can provide the trophic influence, that neither central nor reflex circuitry is required, and that a threshold number of nerves at the amputation surface is needed⁴. The neurotrophic agents do not determine the nature of the regenerate but only its growth properties⁴, and this is borne out by many biochemical studies, all of which suggest that nerves nonspecifically affect macromolecular synthesis and cell division^{5,6}.

Folkman's work on tumour angiogenesis⁷ has emphasised the importance of vascularisation and blood supply in growth processes, and how little we know of the mechanisms involved in angiogenesis in normal development. He has shown that tumours will not grow larger than about 1-2 mm unless vascularised. This suggested to us a new interpretation for the effect of nerves on limb regeneration. Our hypothesis is that the trophic action of the nerves is not on the growth of the regenerate itself but that the nerves are necessary for the vascularisation of the blastema. Thus, early denervation will prevent regeneration by blocking vascularisation.

The vasculature of regenerating limbs of the newt (*Triturus*

Fig. 1 Vascular changes during limb regeneration in the newt *T. cristatus*. *a*, 7 d after amputation. *b*, pre-small cone phase (10 d after amputation): the avascular rim is now about 0.5 mm wide. The whole mounts are difficult to photograph satisfactorily, low lighting being needed to see the otherwise transparent tissues. When viewed end-on, the avascular tip is very clear. *c*, Small cone blastema: this is now beginning to show signs of vascular buds growing into the proximal parts of the blastema. The stump boundary is shown by the arrows. *d*, Flat-cone blastema: the blastema is now fully vascularised and some thicker vessels are beginning to appear. ($\times \sim 9$).

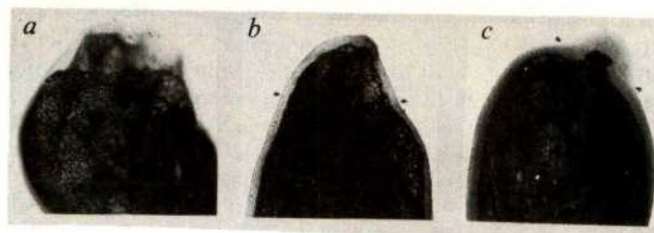
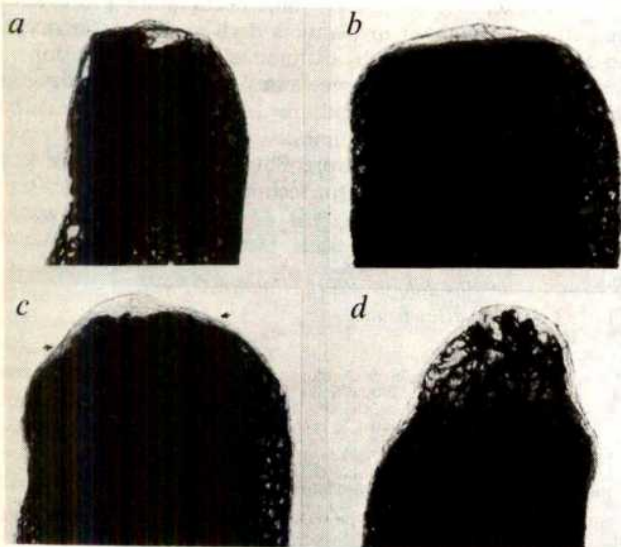


Fig. 2 Vascular pattern of regenerating and of denervated limbs of the axolotl *A. mexicanum*. *a*, 7 d after amputation: this shows clearly the retracted dermal vasculature, leaving a little more than 1 mm of avascular tissue more distally. The fine anastomosing capillary network can be seen easily. *b*, The innervated (control) limb after 17 d of regeneration. *c*, The limb denervated 3 d after amputation and left for 2 weeks to grow (17 d in total). The control side has formed a highly vascularised large blastema (more than 1.5 mm), whereas the denervated side has formed a small avascular blastema (0.5 mm). (The denervated side shows a small haematoma underneath the blastema. This was more common in denervated animals. Whether this is due to fragility of the vasculature or a loss of the ability of vasoconstriction of the ends of the vessels because of denervation is not known.) ($\times \sim 12$).

cristatus) and axolotl (*Ambystoma mexicanum*) was investigated by injecting Indian ink through a fine glass pipette into the hearts of MS222-anaesthetised animals. The limbs were then amputated, fixed in Bouin's, dehydrated and cleared in methyl salicylate. This technique is crude and cannot be expected to reveal subtle changes in the blood supply, but gross changes have been observed. The vasculature of the whole arm can be divided roughly into two main components. The first appears to be situated in the dermis in close proximity to the surface, forming a sheath over the whole arm. It is made up of a very fine anastomosing meshwork of vessels (10-30 μ m diameter), and gives the appearance, in an Indian-ink injected animal, of lace gloves (see Fig. 2a). These vessels can be shown histologically to surround the numerous dermal glands. The second component comprises all the deeper-lying large vessels that supply the other tissues of the arm. These tend to be coarser in appearance except for the fine vessels in the muscles. These are, however, easily distinguishable due to their parallel arrangement. After amputation, the skin contracts away from the cut surface, taking with it the underlying dermal vasculature (Fig. 2a). Thus, in the first few days of regeneration there is a region immediately below the wound epithelium that is avascular. The wound epithelium thickens to form an apical cap and blastema formation starts⁸. The pre-small cone phase is avascular, but by the small cone stage, small capillary buds are beginning to sprout and invade the proximal blastema (Fig. 1). These are fine vessels and can be traced to the dermal network of capillaries as well as the deeper vessels. The distal part of the small cone blastema still appears to be avascular and these tiny vessels do not get to the distal margin until the large cone stage. The avascular early blastema is more striking in the axolotl than in the newt (Fig. 2a). After the large cone stage, bigger vessels are quite often seen in the blastema. The distal margin of the large cone blastema has a continuous capillary supply underneath it. After the large cone stage, this vascular margin becomes fragmented as the delineation of the digits is made, the avascular spaces corresponding to the tips of the future digits. Tubes of vessels form as the indentations mark off externally the four digits. This description is essentially the same as that of Peardon and Singer⁹ for the newt *Triturus viridescens*.

Vascularisation was also investigated in limbs denervated at various stages and at successive intervals after denervation. Both forelimbs were amputated but only one side was denervated, through the brachial plexus, the other serving as a control. If denervation was carried out before the blastema became vascularised, the blastema did not become vascularised during the following 2 weeks whereas the control limbs regenerated normally (Fig. 2b and c). Denervation at the time when the

blastema begins to become vascularised, or later, did not appear to have gross effects on vascularisation but the resultant blood vessels tended to be thicker and the fine dermal network was often absent.

These results are consistent with our hypothesis that vascularisation of the blastema is dependent on the presence of nerves and that vascularisation plays a crucial role in normal regeneration. We can thus envisage the following interactions in regeneration. After amputation the retraction of the dermal vasculature results in an avascular region at the end of the stump. It is this region which undergoes dedifferentiation and gives rise to the blastema. We suggest that the blastema will not grow unless it becomes vascularised and that this vascularisation requires the presence of nerves. This accounts for the behaviour of limbs denervated at an early stage of regeneration. Denervation at stages when the blastema is already vascularised should have no effect on regeneration, unless the continued presence or further growth of the vessels is still nerve dependent. In fact the reduction in growth is associated with a reduction in vascularity. Here, muscle is more likely to be affected than cartilage, and histological investigations have shown this to be so¹⁰. Our hypothesis requires that limbs denervated very early should develop to the stage of a small avascular blastema and this is what both we and Revardel and Chapron¹¹ have found (Fig. 3). Our interpretation also explains why the effects of denervation are nonspecific and simply result in a general

angiogenesis and whether their effect is on the growth and sprouting of vessels themselves, or on tissues they invade. What is clear, is that blood supply and angiogenesis may play a crucial role in limb regeneration.

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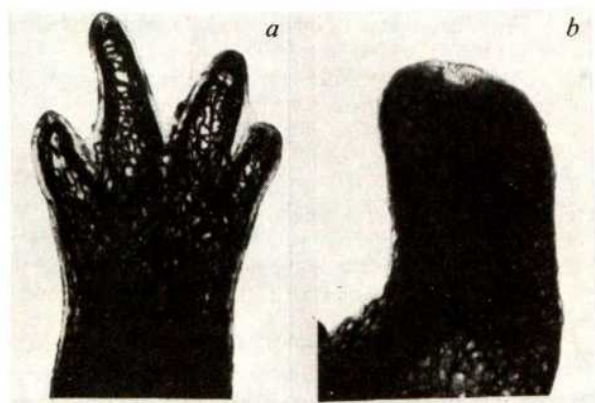


Fig. 3 Vascular pattern of regenerating normal and denervated limbs of the newt *T. cristatus*. *a*, The innervated control limb. *b*, The limb denervated during the pre-small cone phase (about day 10 after amputation) and left to grow for 2 weeks, that is, for the same period as the control. The control side has formed a highly vascularised four digit blastema, whereas the denervated side has remained in the avascular pre-small cone phase. ($\times \sim 15$.)

decrease in cell division and macromolecular synthesis at all stages. Moreover, the transitory increase in cell division and macromolecular synthesis immediately after denervation⁶ could be due to vasodilation caused by denervation. Blood supply may also be involved in the dedifferentiation of stump tissues. Dedifferentiation occurs in the avascular tip and we have shown that bone regression of about 0.5 mm occurs⁸ and this is similar in depth to the avascular region. Revardel and Chapron¹¹ have also emphasised the correlation between dedifferentiation and avascularity. It is also possible that the effects of X irradiation on regenerating limbs are in part a result of effects on the blood vessels.

The idea that blood supply is important in regenerative processes is not new and has been invoked in relation to compensatory hypertrophy of organs such as liver and lung¹²; the novel feature suggested here relates to the role of nerves. While all the above makes our suggestion plausible it must be emphasised that it in no way proves it: it is in fact disconcerting that we have not been able to find any reports relating nerves to angiogenesis in other systems. If we are correct, however, one may begin to consider how nerves could affect

Permeability of solutes through biological membranes measured by a desorption technique

THE influence of molecular structure of a solute on its permeation through biological membranes has been evaluated by various methods^{1–4}. For permeation of a solute across a membrane of thickness, h , the permeability coefficient, P , can be expressed in terms of the membrane-solution partition coefficient (K_m) and the diffusion coefficient in the membrane (D):

$$P = K_m D / h \quad (1)$$

We now present a further method, based on a desorption technique, in which the diffusivity of a solute in a membrane and its membrane-solution partition coefficient can be determined directly. A similar technique has been used in studies of vapour diffusion in polymers⁵. Biological material, initially equilibrated with an aqueous concentration of solute, is immersed in distilled water. The rate at which a solute is desorbed from the membrane reflects its diffusivity whereas the amount desorbed is used in the estimation of the membrane-solution partition coefficient of the solute.

The approach has several advantages. (1) Direct estimation of membrane-solution partition coefficients (which are not affected by the irreversible sorption of the solute on the membrane) avoids analysis of permeation results by organic solvent-water partition coefficients⁶; (2) the diffusivities of different solutes in a membrane are indicated by the rates of desorption of the solutes and may be compared without estimates of membrane thickness; (3) the results of this technique are relatively insensitive to the presence of holes or tears in the membrane; and (4) the presence of complications such as aqueous boundary layer effects⁷ or additional routes of solute penetration^{1–4} may be ascertained by comparison of the permeability estimates from permeation⁸ and desorption experiments.

The several treatments of diffusion (random walk, solution of formal diffusion equations, Eyring's theory and so on) are compatible and lead essentially to the same solution⁹. Crank¹⁰ presented two solutions for the diffusion of a solute into or out of a semi-infinite slab with boundary conditions of in-

stantaneous equilibrium of sorbent entities at the surfaces. The solutions are given as:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[-\frac{(2n+1)^2 \pi^2 D t}{h^2} \right] \quad (2)$$

and

$$\frac{M_t}{M_\infty} = 4 \left(\frac{D t}{h^2} \right)^{1/2} \left[\frac{1}{\pi^{1/2}} + 2 \sum_{n=1}^{\infty} (-1)^n \operatorname{ierfc} \left(\frac{nh}{2(Dt)^{1/2}} \right) \right] \quad (3)$$

where M_t is the amount of solute sorbed or desorbed at time t , M_∞ is the equilibrium amount sorbed or desorbed after infinite time, D is the diffusion coefficient of the solute in the membrane and h is the membrane thickness.

These equations are valid for the desorption of a solute from a biological membrane providing several assumptions are satisfied. (1) There is a single solute in true solution and initially uniformly distributed in the membrane; (2) the composition of the membrane is unaltered during the diffusion process; (3) the diffusion coefficient of the solute is independent of time and position in the membrane, and (4) the solute is desorbed into a well stirred infinite sink which does not affect the desorption process.

If the solute is desorbed from a membrane into a well stirred sink, a contribution to M_t and M_∞ is given by M_i the solute present in solution adhering to the surfaces of the membrane. This may be reduced by rinsing the surfaces of the membrane. In this study the spectrophotometric analytical procedure has introduced a lag time (τ).

Addition of the parameters τ and M_i to the equations yield:

$$M_t = M_\infty - \frac{8(M_\infty - M_i)}{\pi^2} \times \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[\frac{(2n+1)^2 \pi^2 D (t - \tau)}{h^2} \right] \quad (4)$$

and

$$M_t = M_i + 4(M_\infty - M_i) \left(\frac{D(t - \tau)}{h^2} \right)^{1/2} \times \left[\frac{1}{\pi^{1/2}} + 2 \sum_{n=1}^{\infty} (-1)^n \operatorname{ierfc} \left(\frac{nh}{2D^{1/2}(t - \tau)^{1/2}} \right) \right] \quad (5)$$

The amount desorbed (M_t) is nonlinear with time (t) with four unknown parameters τ and M_∞ , M_i and D/h^2 . Since approximate values of τ and M_∞ are known, estimates for M_i and D/h^2 may be found using an approximation of equation (5) which applies over short times:

$$M_t = M_i + 4(M_\infty - M_i) \left(\frac{D(t - \tau)}{\pi h^2} \right)^{1/2} \quad (6)$$

With these initial estimates, an iterative nonlinear least squares regression program (NONLIN, C. M. Metzler, Upjohn, which uses a modification of Hartley's¹ method to estimate the parameters of a system of nonlinear functions) was used. The best parameter values were found by minimising equation (7) until convergence (where lack of change in sums of squares (SS) is the convergence criterion):

$$SS = \sum_{i=1}^n \left(M_{t_i \text{ observed}} - M_{t_i \text{ predicted}} \right)^2 \quad (7)$$

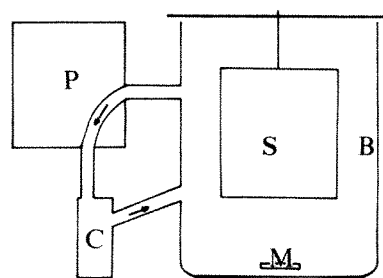


Fig. 1 Arrangements for monitoring the desorption of a compound from a biological membrane. Compound is desorbed from the membrane held in mesh support, S, into thermostated beaker, B, containing distilled water and stirred with a magnetic stirrer, M. The desorbing solution is pumped at a constant rate through the peristaltic pump, P, directly into a thermostated flow-through spectrophotometer cell, C, back into the beaker. M_t , the amount desorbed at time, t , is estimated from the observed absorbance, A_t , of the desorbing solution. (For desorption into 60 ml, suitable absorbance values have been obtained using 5–25 mg wet weight of stratum corneum.)

Equation (4) was evaluated satisfactorily using four exponential terms and additional terms gave no improvement. A polynomial was used to evaluate the error function series and the summation term in equation (5) was evaluated until the change in its value was less than 1×10^{-5} , which is insignificant compared with $1/\pi^2$. A typical regression curve for a set of data is shown in Fig 2. Parameter estimates are independent of whether equation (4) or (5) is used.

The solute membrane-solution partition coefficient (K_m) may be evaluated from the expression

$$K_m = \frac{(M_\infty - M_i)}{W_m C_s}$$

where W_m is the weight of the membrane, C_s is the concentration of the solute in the solution with which the membrane was equilibrated and $(M_\infty - M_i)$ is the amount of solute desorbed. Some stratum corneum-water partition coefficients are shown in Table 1.

A Collander-type plot¹² of the stratum corneum-water partition coefficient (K_m) against the octanol water-partition coefficient (K_o) shows good correlation (Fig. 3). The values of D/h^2 are indicative of the diffusivities of solutes in a membrane and those for several phenols are listed in Table 1. Good reproducibility of K_m and D/h^2 was found on repeated use of the same membrane, the use of samples of stratum corneum taken from the same area of a cadaver, and initial equilibration of the membrane with different concentrations of solute.

Fig. 2 Typical regression curve for observed absorbance against time following desorption of a solute (*p*-cresol) from hydrated stratum corneum. Correlation coefficient = 0.998; $(D/h^2) \times 10^3 = 1.46 \pm 0.08 \text{ s}^{-1}$; $(A_\infty - A_i) = 0.117 \pm 0.003$; $A_i = 0.010 \pm 0.001$; $\tau = 7.15 \pm 0.15 \text{ s}$; sum squared observations = 0.264; sum of squared deviations (SS) $\times 10^5 = 0.500$.

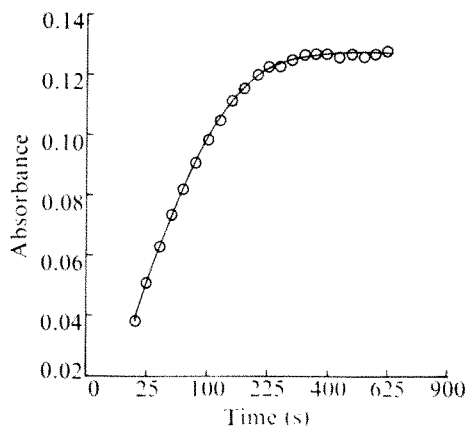


Table 1 Desorption data for human stratum corneum

Solute	$K_m \pm \text{s.d.}$	$D/h^2 \pm \text{s.d.}$ ($\text{s}^{-1} \times 10^3$)	Regression correlation coefficient
Benzyl alcohol	5.0 ± 0.2	0.88 ± 0.04	0.997
Phenylethyl alcohol	5.4 ± 0.3	1.01 ± 0.09	0.992
Phenol	5.4 ± 0.2	0.98 ± 0.05	0.996
<i>p</i> -Cresol	10.3 ± 0.2	1.46 ± 0.08	0.998
<i>m</i> -Cresol	10.4 ± 0.3	1.30 ± 0.07	0.995
<i>o</i> -Cresol	10.5 ± 0.2	1.21 ± 0.08	0.994
<i>p</i> -Bromophenol	27.7 ± 0.4	1.10 ± 0.06	0.997

The wet weight of stratum corneum (separated from other epidermal layers¹⁴) may be estimated by a dehydration technique¹⁶. Combination of D/h^2 , h , and K_m may be used to estimate P using equation (1).

This technique seems to be applicable to studies of drug absorption, pharmaceutical formulation and membrane transport. In the study of drug administration and disposition, the diffusivity and partition behaviour of solutes in various mammalian tissues are of interest.

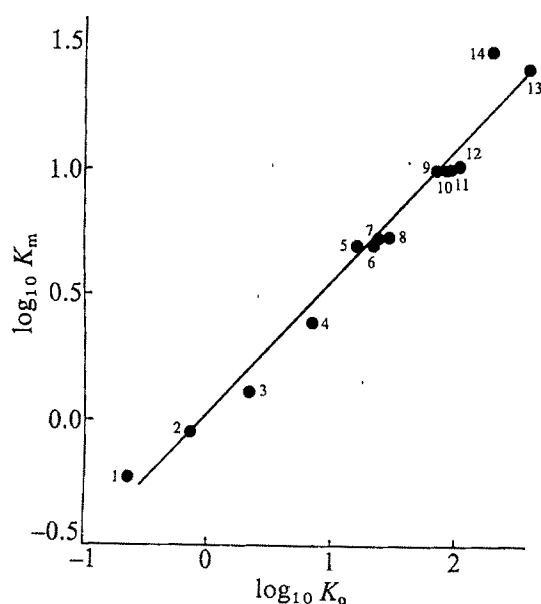


Fig. 3 Collander-type plot of stratum corneum-water partition coefficient (K_m) against octanol-water partition coefficient (K_o)^{3,13} for some non-electrolytes. Compounds are referred to as follows: 1, methanol; 2, ethanol; 3, propanol; 4, butanol; 5, benzyl alcohol; 6, pentanol; 7, phenylethyl alcohol; 8, phenol; 9, hexanol; 10, *p*-cresol; 11, *m*-cresol; 12, *o*-cresol; 13, *p*-bromophenol; 14, heptanol. (Stratum corneum-water partition coefficients are from Table 1 and ref. 4.)

Corneal epithelium (50 μm), stratum corneum (10 μm) and dermis (1,000 μm) are of suitable thickness for evaluation by this technique. For very thin membranes, the technique would have only limited application because desorption would occur rapidly. Since spectroscopy, radiotracer methods, conductometry and so on are suitable analytical methods for this technique, a wide range of solutes may be investigated.

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Macrophages on intrauterine contraceptive devices produce prostaglandins

THE mechanisms whereby intrauterine contraceptive devices (IUDs) exert their anti-fertility effects remains unestablished¹. The involvement of prostaglandins (PGs) is being investigated²⁻⁵, although alternative mechanisms have been proposed (for example, phagocytic and other actions of macrophages^{6,7}). Although the relative contributions of macrophages and PGs to the anti-fertility actions of IUDs remains uncertain, it is clear that introduction of the IUD into the endometrial cavity results in an infiltration by neutrophils and macrophages⁸ as well as increased PG levels in uterine venous blood in animals^{9,10}. While investigating the role of mediators of chronic inflammation we have demonstrated that the macrophage is a potent source of PGE₁¹¹, and since the macrophage is the predominant cell adhering to the IUD⁸ we have considered the possibility that such cells act as a source of PGs.

IUDs routinely removed at out-patient clinics were incubated in 10-20 ml Eagle's MEM supplemented with 10% heat-inactivated foetal calf serum for 24 h at 37 °C in an atmosphere of 5% CO₂ in air. After incubation, supernatants were centrifuged and stored at -20 °C for PG assay. Cells were removed from the devices by treatment with 0.01% trypsin for 5 min at 37 °C, and total cells counted with cell viability established by dye exclusion. Differential cell counts were made on cell smears stained with Toluidine blue, Papanicolaou or Giemsa stains. PG concentrations were determined by radioimmunoassay¹² using sheep antibodies to PGE₂/bovine serum albumin and to PGF_{2 α} /bovine serum albumin. Anti-PGE₂ antiserum did not distinguish PGE₁ from PGE₂ (cross reaction 100%); the relative cross reactivities of other PG groups, based on the amount of unlabelled PG required to displace 50% antibody-bound label, were PGF_{2 α} (13%), PGA₂ (1.3%) and PGB₂ (< 0.6%). Anti-PGF_{2 α} antiserum (cross reaction of PGF_{2 α} 100%) also cross reacted with PGF_{1 α} (27%), but only slightly with PGE₂ (< 0.13%), PGA₂ (< 0.013%) and PGB₂ (< 0.016%). Results were expressed as PGE₂ or PGF_{2 α} equivalents. Seventeen IUDs have been examined so far including eleven of the type Gravigard Copper 7 (Searle, UK), four Lippes loops (Ortho, USA), and two Dalkon shields (Robins, USA). In all cases significant PG activity has been detected in culture fluids collected at 24 h (Table 1). The time course of PG production was determined for several devices

Table 1 PG production by IUDs in *in vitro* culture

IUD (n)	Total WBC ($\times 10^7$) mean \pm s.e.m.	Prostaglandin-like activity in culture fluid (ng PG eq. per 10^7 cells per 24 h) mean \pm s.e.m.	
		PGE ₂	PGF _{2α}
Copper 7 (11)	2.25 ± 0.67	190 ± 52	464 ± 110
Lippes loop (4)	2.00 ± 0.78	161 ± 107	287 ± 198
Dalkon shield (2)	7.50 ± 2.9	92 ± 38	249 ± 37

IUDs cultured in Eagle's MEM and 10% foetal calf serum for 24 h at 37 °C in a 5% CO₂ in air atmosphere. Cells removed by centrifugation and counted. Supernatants stored for assay. 50- μl volumes assayed for PGE₂ and PGF_{2 α} activities by radioimmunoassay. WBC, White blood cells; n, number of IUDs.

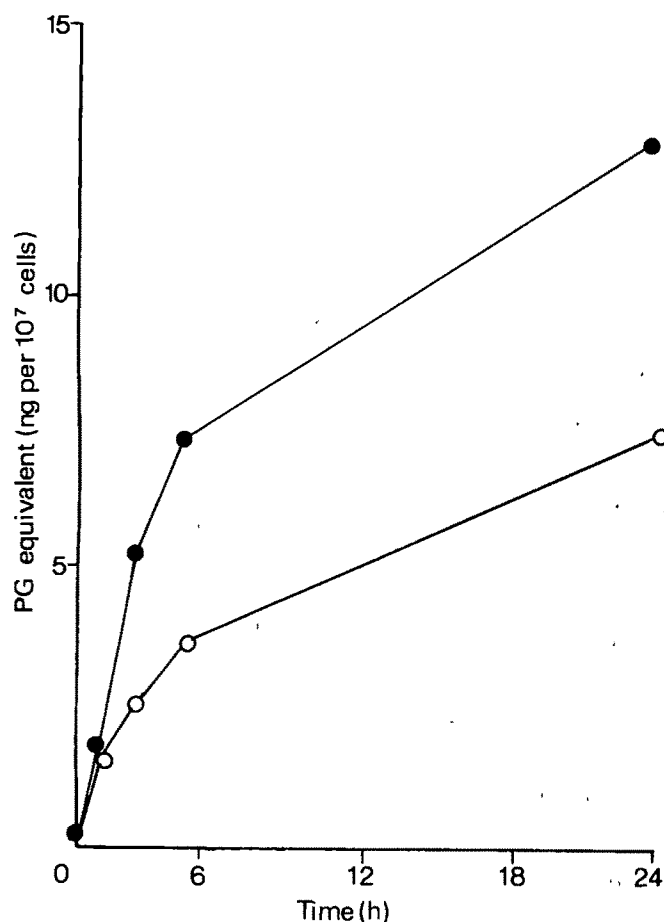


Fig. 1 Time course of prostaglandin (PG) generation for a Copper 7 IUD. The ordinate depicts the concentration of PGE₂ (○) and PGF_{2α} (●) expressed as ng per 10⁷ cells as determined by radioimmunoassay.

and Fig. 1 shows a time course for PGE₂ and PGF_{2α} production by a Copper 7. In general, the PG production correlated with the total cell number on the IUD, and production of PGF rather than PGE predominated in 16 of the 17 devices examined. This contrasts with the guinea pig macrophage where the PGFs constitute less than 10% of the PG activity¹¹ and may relate to the close proximity of macrophages with the copper component of the Copper 7 device, since copper modifies PG production in other situations^{13,14}, or to the regulatory actions of steroid hormones on macrophages¹⁵. Cells identified morphologically as macrophages were the principal cell type on each device. Substantial numbers of neutrophils were observed on some devices but were not associated with enhanced PG production, and in view of the comparative ineffectiveness of neutrophils in PG generation compared with macrophages^{11,16-18}, it seems unlikely that the neutrophil is the source of the PG activity associated with IUDs.

These observations relate two lines of evidence concerning the suggested mode of action of IUDs by showing that the macrophages adherent to IUDs are a likely source of the increased PG concentrations associated with these contraceptive devices. Because the macrophage is such a potent source of prostaglandin, it is worth considering this cell as a source of uterine prostaglandins. Macrophages show cyclical changes in activity in response to altered circulating steroid levels during the oestrous cycle¹⁵, and thus may provide a mechanism whereby the changes in steroid levels can determine the variations in PG production by the uterus during the oestrous cycle¹⁹.

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Cytocidal and cytostatic effects of activated peritoneal leukocytes

MACROPHAGES seem to be of importance in both immunologically specific and nonspecific resistance to neoplastic cells¹⁻¹⁰. It has been proposed¹¹⁻¹³ that activated macrophages are significant effectors in the elimination of tumour cells at sites of delayed hypersensitivity reactions induced by chemical haptens, microbial antigens or lymphokines¹³⁻¹⁸. Immunologically specific elimination of neoplastic cells by macrophages may be mediated by tumour-specific antibodies^{7,10,19,20}. Such antibodies may also confer specificity to macrophages activated by nonspecific stimulation. Besides specificity which may be imparted by antibodies, activated macrophages seem to possess an inherent recognition mechanism which permits selective cytotoxicity towards certain categories of cells, including tumour cells^{11,21-23}.

Although it seems that activated macrophages are cytotoxic towards neoplastic cells^{8,9,11,12,21-23}, there are seemingly conflicting reports on the effects of activated macrophages on cells established from normal tissues. In a number of *in vitro* studies^{11,21-23} insignificant or no effects of activated mouse or rat peritoneal macrophages were noted on fibroblasts of normal adult or embryonic tissue origin. Normal mouse embryonic fibroblasts have, however, been reported to be destroyed by peritoneal leukocytes of nonspecifically stimulated mice²⁴. Our present results offer a possible reconciliation of these differing observations. Rat embryonic cells of a low *in vitro* passage number and a concomitant high rate of replication were found to be susceptible to cytotoxic effects of peritoneal leukocytes of nonspecifically stimulated syngeneic rats. In contrast, embryonic cells of a higher *in vitro* passage number and a lower rate of replication were relatively resistant to such leukocyte preparations.

Rats of the inbred Fischer strain were used. Cells of whole rat embryos close to term were stored in liquid nitrogen. In any one experiment the embryo cells were from the same batch in the second (FE₂) or the sixth (FE₆) *in vitro* passage. Sarcomas were induced by injection of newborn rats with polyoma virus. Cells of these tumours (PYT) were established *in vitro*. Peritoneal leukocytes were collected by intraperitoneal lavage of rats which 48 h previously had received an intraperitoneal injection of 50 µg PPD (purified protein derivative of tuberculin). The differential counts of the peritoneal cells were: large mononuclear cells, 65-75%; small lymphocytes, 10-15%; neutrophils,

Table 1 Effect of challenge of PYT, FE₂ and FE₆ cells with peritoneal leukocytes from PPD-stimulated Fischer rats

Culture combination	Target cells per dish ($\times 10^3$) at time 0	Target cells per dish ($\times 10^3$) at 48 h	Unchallenged cultures (%)	Growth index
PYT+M	241; 217	1,747; 1,652	100	7.5
PYT+SP		75; 62	4; 4	
FE ₆ +M	115; 105	188; 182	100	1.7
FE ₆ +SP		144; 141	77; 76	
FE ₂ +M	140; 157	552; 545	100	3.7
FE ₂ +SP		138; 136	25; 25	

Duplicate cultures of PYT or rat embryo cells in 2nd or 6th *in vitro* passage were challenged with peritoneal leukocytes (SP) of a PPD-stimulated rat. Companion cultures received medium (M) only. After 48 h the cells were collected and the target cells counted electronically. The growth index is the ratio between cell counts at 48 h and time 0 of unchallenged cultures.

4–12%; eosinophils, 4–8%, mast cells, 4–8%. Cytotoxicity of the peritoneal leukocytes towards the various target cells was tested as follows. Target cells were seeded in 35-mm round plastic dishes 24 h before use and incubated at 37 °C in a water-saturated atmosphere containing 5% CO₂. On the day of initiation of an experiment (time 0) the culture fluid was removed and replaced with 3 ml of either fresh medium or a suspension of 2×10^6 peritoneal leukocytes. After a further 48-h incubation the cells of duplicate cultures of the various experimental groups were collected and the target cells counted electronically. These procedures, as well as the basis for the use of PPD stimulation of the peritoneal leukocyte donors, have been described previously^{23,26}.

In a series of four experiments, cultures of PYT, FE₆ and FE₂ cells were challenged with peritoneal leukocytes from PPD-stimulated Fischer rats. A set of companion cultures remained unchallenged. The data from one such experiment are presented in Table 1; comparable figures were obtained in the other three experiments. The peritoneal leukocytes substantially reduced the numbers of the sarcoma cells (Table 1). The final relative counts of these cells were of the order of 4% of the unchallenged companion cultures and their absolute numbers were significantly lower than the time zero counts. In contrast to the sarcoma cells, FE₆ embryo cells increased in numbers when cultured with the peritoneal cells, reaching 76–77% of the cell numbers of the unchallenged controls. The numbers of the FE₂ embryo cells, on the other hand, remained essentially stationary when challenged with the same peritoneal leukocyte preparation. These findings suggest that susceptibility to the cytotoxicity of activated peritoneal macrophages is not solely an attribute of neoplastic cells. The effect of the activated peritoneal leukocytes on the low passage embryonic cells, however, had more the character of growth inhibition than the frankly cytotoxic effect on the sarcoma cells.

Previous observations^{11,21–23} have indicated that susceptibility or resistance of cells to the cytotoxicity of activated peritoneal leukocytes is not likely to be related to recognition of target cell antigenic determinants, nor is it a consequence of non-specific culture conditions, such as crowding of cells or metabolic depletion. Table 1, together with other findings^{23,26}, suggests an association between growth rate of normal embryo cells and susceptibility to the cytotoxicity of nonspecifically activated peritoneal leukocytes. Of possible relevance to this association is the agglutinability of non-transformed 3T3 mouse cells by plant lectins only when the cells were in mitosis²⁷. The same 3T3 cells after transformation by polyoma virus, however, were agglutinated by the lectins at any time, irrespective of their position in the growth cycle. It seems therefore that transformed cells continuously exhibit surface properties which normal cells only exhibit when they are in mitosis. Such or similar properties are possibly also reflected in the differential cytotoxic effects reported here. The mechanism of the cytotoxic and the cytostatic effects of activated macrophages may not be fundamentally different. In both situations

destruction of susceptible target cells is likely to take place. The final outcome of macrophage interactions with normal cells in terms of changes in target cell numbers in given culture conditions with respect to macrophage and target cell densities, however, may depend on the relative numbers of target cells in mitosis.

An alternative interpretation of the difference in susceptibility of the high and low passage embryonic cells is selection of a relatively resistant population during *in vitro* cultivation. When initially prepared, embryo cells represent a very heterogeneous population. Certain segments of this population may be susceptible, whereas others may be resistant to the cytotoxicity of activated peritoneal leukocytes. After a number of passages *in vitro* a change in the proportions of these segments may take place resulting in a population with a higher overall resistance.

Neoplastic cells may have certain properties in common with embryonic cells. An additional consideration, therefore, is that the apparent difference in susceptibility of embryo cells of low and high *in vitro* passage number could be attributable to cellular differentiation during cultivation *in vitro*. At the time of isolation from the embryos, the cells may exhibit certain surface properties which are lost during growth *in vitro* as a sequel to differentiation. Although macrophages are present in noticeable numbers in some embryonic tissues at certain stages of differentiation²⁸ a possible relationship of the observations reported here to a specific function of macrophages in embryonic development is at present conjectural.

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Partial characterisation of Ia antigens on murine thymocytes

GENES controlling specific immune responsiveness at the level of activation of the thymus derived (T) lymphocyte, and collaboration of these cells with lymphocytes derived from the bursal equivalent (B lymphocytes), have been shown to be linked to the major histocompatibility complex (MHC) in many species¹. The mechanism of action of these genes is not understood, and it has been postulated that they represent structural genes for a non-immunoglobulin receptor for antigen on T lymphocytes².

In the mouse, the immune response (Ir) genes linked to the MHC have been mapped to the I region of the H-2 complex³. The I region has recently been shown to be associated with antigenic molecules (Ia antigens) expressed predominantly on B lymphocytes, but also on several non-lymphoid populations. The Ia antigens might represent products of the Ir genes. Paradoxically, in view of the strong evidence for the expression of the MHC-linked Ir genes in T cells¹, whether or not Ia antigens are expressed on T cells has been a matter of some controversy⁴⁻¹⁰. Because of the continuing debate on the nature of the T-cell receptor^{11,12}, the question of the expression of Ia antigens on T lymphocytes is of considerable importance. Previous work from this and other laboratories has indicated that Ia antigens can be detected on T lymphocytes^{8,10} and T lymphoma cells⁹. Ia antigens from several cell sources have also been partially characterised by immunochemical techniques^{8,13,14}, although so far attempts to isolate Ia antigens from thymocytes have proved unsuccessful. In this report, we demonstrate the specific immunological precipitation of Ia antigens from extracts of surface radioiodinated thymocytes.

The outer membranes of thymocytes from A.TL mice were radioiodinated by the lactoperoxidase technique¹⁵ and extracted with the non-ionic detergent Nonidet P-40 (NP-40). The cell extract was then held with A.TH anti-A.TL (anti-I^a; experimental) serum or A.TL anti-A.TH (anti-I^a; control) serum, followed by rabbit anti-mouse IgG serum. The resulting precipitates were centrifuged, washed, and analysed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) and mercaptoethanol¹⁶.

As shown in Fig. 1, radioiodinated surface immunoglobulin was not detected in these conditions. It has previously been shown that NP-40 extraction does not allow clear demonstration of immunoglobulin on murine thymocyte membranes, although it is very efficient in extraction of immunoglobulin from B lymphocytes¹⁷. In our first experiments, a large peak of radioactivity migrating a little ahead of the γ chain in both control and experimental precipitates was found. It was precipitable by IgG-anti-IgG, IgM-anti-IgM and fowl IgG-anti-fowl IgG complexes, and was invariably present in comparable amounts in control and experimental precipitates. This peak has been observed elsewhere^{18,19}, and was considered to be a component which binds to antigen-antibody complexes. In subsequent experiments, this component was depleted by a factor of approximately five by preliminary precipitation with fowl IgG and rabbit anti-fowl IgG (legend to Fig 1). In addition, a small but unequivocal peak of radioactivity migrating just behind the light chain standard was consistently observed in the experimental (A.TH anti-A.TL) but not in the control (A.TL anti-A.TH) precipitates. Identical results were obtained in three independent experiments, and the peak was also clearly visible in autoradiographs. This peak corresponded to a molecular weight of approximately 30,000, although determination of molecular weight by polyacrylamide gel electrophoresis must be made with some caution, as carbohydrate differences considerably influence mobility²⁰. A third peak, corresponding to an R_f of approximately 0.05, was seen in some experiments. It was rather variable in size, but was

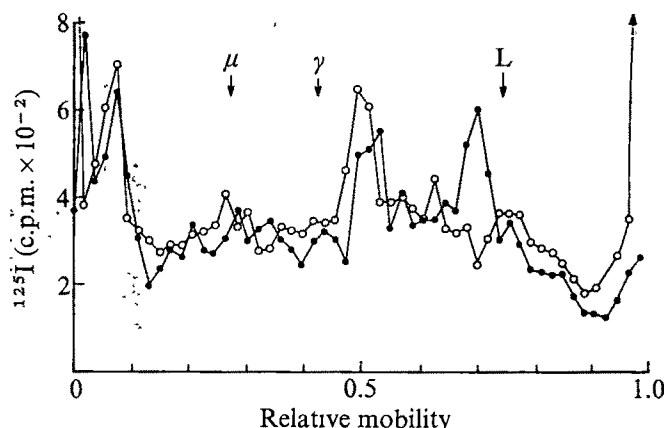


Fig. 1 Thymuses from 6-week-old female A.TL mice were removed, taking care to avoid removal of the parathyroid lymph nodes, and teased gently through a fine sieve into phosphate-buffered saline (PBS). Dead cells and clumps were removed by the method of von Boehmer and Shortman²¹. Cells were surface radioiodinated with ¹²⁵I as follows. To five plastic centrifuge tubes, each containing 10⁷ cells in 30 μ l PBS, was added 10 μ l lactoperoxidase (0.25 mgml⁻¹), 5 μ l carrier free ¹²⁵I sodium iodide (0.05 mM, 100 μ Ci μ l⁻¹), and 10 μ l 8.8 mM H₂O₂. Cells were held at 30 °C for 5 min, pooled, and washed once with 5 ml PBS. They were then extracted with 1% NP-40 in PBS (3.5 ml) at room temperature for 1 h, centrifuged, and the supernatant dialysed against PBS for 4 h at 4 °C. The extract was then centrifuged at 10,000g for 30 min, and the supernatant divided into 200- μ l aliquots, to which were added 100 μ l rabbit anti-fowl IgG (absorbed against mouse immunoglobulin) and 100 μ l of 0.1 mgml⁻¹ fowl IgG. The mixture was held at 37 °C for 1 h, and 4 °C overnight. It was then centrifuged at 10,000g for 20 min, and the precipitates discarded. To each tube was added 2 μ l A.TH anti-A.TL serum, or A.TL anti-A.TH serum prepared as previously described⁶. Both antisera were extensively absorbed for autoantibodies before use. The extracts were incubated at 37 °C for 1 h, and then an appropriate amount of rabbit anti-mouse IgG serum added quantitatively to precipitate the mouse IgG. A further incubation was performed at 37 °C, and the tubes left at 4 °C overnight. The precipitates were washed four times, and then boiled in a buffer solution containing 5% mercaptoethanol and 3% SDS, and analysed by discontinuous polyacrylamide gel electrophoresis as described by Laemmli and Favre¹⁶. The total concentration of polyacrylamide was 10%, and the concentration of bisacrylamide was 0.25%. Gels were 10 cm long and 5 mm in diameter. The precipitated material from 5 \times 10⁷ cells was ultimately distributed among four gels (two control and two experimental), each gel thus representing material from 1.25 \times 10⁷ cells. Gels were sliced transversely into 2-mm sections and counted in a Packard gamma counter, or sliced longitudinally and processed for autoradiography. Calibration of molecular weights was done by running parallel gel electrophoresis of proteins of known molecular weights. ●, A.TH anti-A.TL serum; ○, A.TL anti-A.TH serum.

always of similar height in experimental and control precipitates. This peak may represent high molecular weight aggregates.

It is unlikely that the 30,000 molecular weight peak is due to the presence of small numbers of B lymphocytes in the thymocyte cell suspension. The fraction of A.TL thymus cells with high density surface immunoglobulin was found to be less than 0.5%, as assessed by autoradiography using polyspecific radioiodinated anti IgM serum, and we are unable to detect Ia extracted from this number (6 \times 10⁴) of B lymphocytes. Further, it has been demonstrated that the TCA precipitable radioactivity incorporated into NP-40 extractable immunoglobulin on B cells following surface iodination is considerably more than that incorporated into B cell Ia antigens¹⁴, yet no radioactivity corresponding in mobility to immunoglobulin chains was seen (Fig. 1). Thus, the relative amounts of Ia and immunoglobulin do not support the hypothesis that the detected Ia was obtained from B cells.

There are several possible reasons for our ability to extract and identify Ia from thymocytes. First, and probably of prime importance, is the titre and specificity of the anti-Ia serum used. The A.TH anti-A.TL serum was of very high titre (1 : 2,000

against spleen cells by semi-microcytotoxicity and 1 : 10,000 by microcytotoxicity tests using rabbit complement), and has been shown lightly but specifically to label 10–20% of thymocytes in autoradiographic experiments⁸. Fathman *et al.* (C. G. Fathman, J. L. Cone, S. O. Sharrow, H. Tyrer, and D. H. Sachs, personal communication) have also shown, using the fluorescence activated cell sorter, that a subpopulation of thymocytes bears Ia antigens in small amounts. Many anti-Ia sera, usually of much lower titre, only react with B cells⁴. Second, labelling by surface radioiodination, rather than biosynthesis using radiolabelled amino acids, allows a far better signal to noise ratio, since radioactivity is confined to the area of interest. Third, optimal iodination conditions and excellent cell viability, both before and after iodination, were found to be essential for good results.

Our results are consistent with the serological data indicating that Ia antigens are present in large amounts on B lymphocytes and in much smaller amounts on T lymphocytes^{8,10}. The sera used in our studies potentially recognise the products of all I subregions. In view of the increasing evidence for heterogeneity of I region products, future work must concentrate on the use of sera of more restricted specificity, and attempt to understand the possible relationships between the Ia antigens and products of the immune response genes.

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Isolation and properties of a murine spleen cell Fc receptor

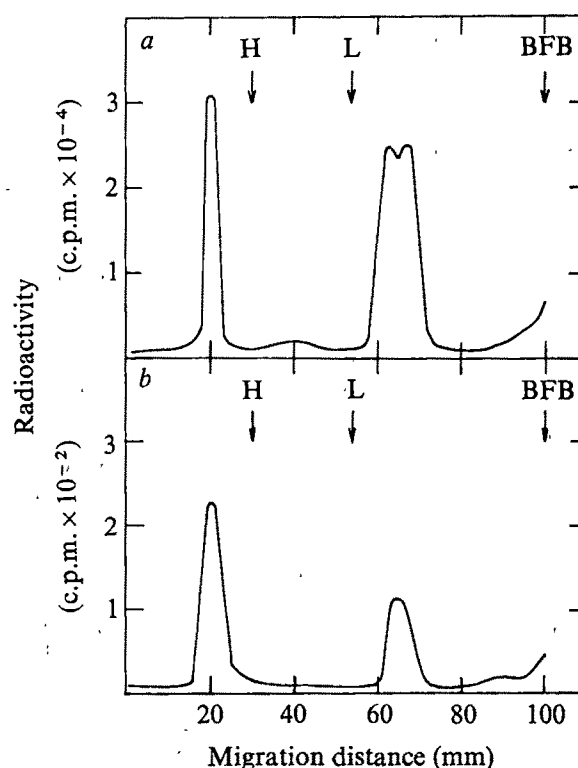
MURINE and human bone marrow-derived (B) lymphocytes have a cell surface-bound receptor for antibody complexed to antigen. Heat-aggregated immunoglobulin complexes will also bind to this receptor, and the binding is specifically mediated by means of the Fc portion of the IgG molecule^{1–3}. So far, the only function ascribed to the Fc receptor is in the antibody-dependent lymphocyte-mediated cytotoxicity reaction⁴. A recent report has indicated, however, that the Fc receptor is identical to or closely associated with the Ia antigens on the B-cell membrane⁴. The Ia antigens are alloantigens coded for by the immune response region of the major histocompatibility

complex⁵ and if indeed these antigens and the Fc receptor are identical, this would reveal a role for the receptor in several aspects of the immune response⁶.

To gain insight into the possible relationship between the Fc receptor and the Ia antigens it seems mandatory to isolate and characterise chemically these molecules. As a first step towards this goal we have isolated an Fc receptor from mouse spleen cells and here report some of its properties.

Crude membrane fractions were isolated from 400 A/Sn mouse spleens. The membrane fraction was suspended in 0.02 M Tris-HCl buffer, pH 7.4, containing 80 mM EDTA and 50 mM β -mercaptoethanol at a final protein concentration of 10 mg ml⁻¹. Soluble macromolecules were recovered after centrifugation of the incubation mixture at 100,000g for 60 min. The supernatant was subsequently subjected to affinity chromatography on a column of Sepharose 4B to which heat-aggregated human IgG had been covalently attached. The column was eluted as described⁷. The eluted macromolecules were labelled with ¹²⁵I and subjected to sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis⁷ together with suitable marker proteins of known molecular weight. Figure 1 depicts a typical result of an electrophoretic separation in SDS of ¹²⁵I-labelled macromolecules with affinity for heat-aggregated IgG. Three types of polypeptide chains with apparent molecular weight of approximately 65,000, 18,000 and 15,000 were clearly distinguishable. Figure 1 also demonstrates that the 65,000-dalton component as well as one (or both) of the two smaller polypeptide chains contained sialic acid as evidenced by radioactive labelling with ³H-sodium borohydride⁸. The three ¹²⁵I-labelled polypeptide chains were extensively reduced and alkylated in 6 M guanidine hydrochloride and subjected to gel chromatography on a column of Sepharose 6B equilibrated with the same solvent. The elution

Fig. 1 SDS–polyacrylamide gel electrophoresis of ¹²⁵I-labelled (a) and ³H-sodium borohydride-treated (b) highly purified mouse spleen cell Fc receptor. The macromolecules were passed over a Sepharose 4B-coupled heat aggregated IgG column. Material attached to the column was eluted with 0.05 M sodium citrate buffer, pH 3.0, containing 0.5 M NaCl. The recovered protein, labelled with ¹²⁵I or ³H, was introduced into sialic acid residues. H and L denote the migration positions of marker heavy and light chains of IgG. The marker dye was bromophenol blue (BFB).



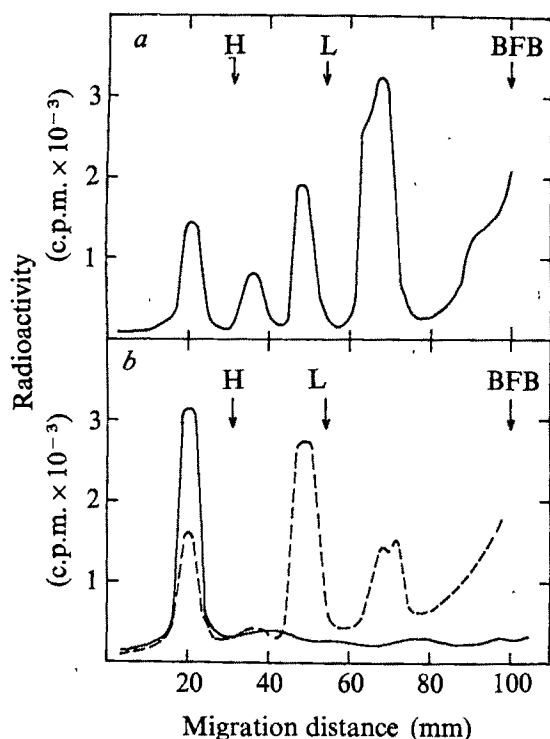


Fig. 2 SDS-polyacrylamide gel electrophoresis of ^{125}I -labelled highly purified mouse spleen cell Fc receptor. *a*, The material was the same as shown in Fig. 1 but the electrophoresis was performed after 2 d incubation at 4°C . *b*, The 65,000-dalton component after thermolysin. The ^{125}I -labelled 65,000-dalton component was mixed with bovine IgG and digested with thermolysin (20% w/w) for 10 min at 37°C . —, Material before proteolysis; — — —, after thermolysin digestion.

positions of the ^{125}I -labelled molecules corresponded to apparent molecular weights of 65,000, 14,000 and 13,000, respectively. The higher molecular weight values obtained by SDS-electrophoresis are consistent with the presence of carbohydrate in the Fc-receptor proteins. This strongly suggests that each component represents a single polypeptide chain.

The specificity and efficiency of the isolation procedure was investigated by incubating ^{125}I -labelled samples of the crude membrane protein and of the breakthrough material from the affinity chromatography column with antigen-antibody complexes. The antigen-antibody complexes were formed from bovine serum albumin (BSA) and a heat-inactivated rabbit antiserum against BSA. After 30 min of incubation, precipitates were collected by centrifugation, extensively washed and subjected to SDS-polyacrylamide gel electrophoresis. The electrophoreses revealed that the starting material contained the same three components which had been isolated by affinity chromatography, but the smaller polypeptide chains were now the dominant components. The absorption on to the affinity chromatography column was very efficient since the breakthrough material recovered from the column contained negligible amounts of protein reacting with the antigen-antibody complexes.

Similar experiments showed that the isolated Fc-receptor components retained their affinity for antigen-antibody complexes even after desorption from the affinity chromatography column.

The three polypeptide chains were encountered in all highly purified preparations. On standing at 4°C for a few days additional components arose, presumably because of the presence of proteolytic activity in the preparations. Figure 2*a* depicts an SDS-polyacrylamide gel electrophoresis of the same material as shown in Fig. 1, but after 2 d at 4°C . It can be seen that in addition to the 65,000, 18,000 and 15,000-dalton components, new material appeared in the apparent molecular weight positions 30,000 and 45,000, respectively. This result

suggested that the 18,000 and 15,000-dalton components were degradation products of the 65,000-dalton molecule. That this indeed is the case was demonstrated by subjecting the isolated 65,000-dalton component to thermolysin digestion. Figure 2*b* shows that such treatment gave rise to three additional polypeptide chains. The main component had an apparent molecular weight on SDS-polyacrylamide gel electrophoresis of about 30,000, whereas the two smaller components had molecular weights of 18,000 and 15,000, respectively. Solubilisation of membrane macromolecules by treatment with NP-40 or by papain digestion usually gave Fc-receptor components with apparent molecular weights of about 30,000 and 15,000 as estimated on SDS-polyacrylamide gel electrophoresis. A common origin for the 65,000, 18,000 and 15,000-dalton components was also suggested by the immunological cross reactivity between the three polypeptide chains (L.K., L.R. and P.A.P., unpublished).

Rabbit antisera were raised separately against the 65,000-dalton material and the mixture of the 18,000 and 15,000-dalton components. Table 1 shows that such antisera reacted exclusively with B lymphocytes. That the antisera bound to the B-cell Fc receptor was documented in a separate experiment. Aggregated IgG was labelled with fluorescein isothiocyanate³ (FITC) and allowed to bind to spleen cells treated with various antibody IgG and (Fab')₂ fractions. Table 2 shows that intact antibodies of various specificities all impeded the binding of aggregated IgG provided they bound to the B-cell surface. This result is in agreement with previous findings⁹. When (Fab')₂ fragments, lacking the Fc portion, of these antisera were used, however, only those directed against the isolated 65,000-dalton and the 18,000–15,000-dalton components were effective (Table 2). The (Fab')₂ fragments against H-2^k were also effective in reducing the number of cells containing aggregated IgG. This antiserum retains its ability to abolish binding of aggregated IgG to B cells even after all measurable anti-H-2 alloantigen activity has been absorbed out on thymocytes of the relevant antigen type. It seems reasonable to conclude that the antiserum contains anti-Ia antibodies since similar results were obtained with a specific anti-Ia serum (Table 2)^{4,9}.

If the Fc receptor and the Ia antigens are physically associated

Table 1 Cell binding of antibodies against the 65,000-dalton polypeptide chain and against the mixture of the 18,000 and 15,000-dalton components

Cell source	Treatment*	Antiserum	Stained cells (%)
Spleen	—	65,000	44 ± 6
Spleen	—	18–15,000	42 ± 8
Spleen	anti-Thy1 2	65,000	86 ± 4
Spleen	anti-Thy1 2	18–15,000	81 ± 11
Lymph nodes	—	65,000	22 ± 2
Lymph nodes	—	18–15,000	20 ± 3
Spleen	—	Ig	42 ± 5
Lymph nodes	—	Ig	16 ± 6

The cells obtained from C₃H/He mice were adjusted to a final concentration of 5×10^7 cells per ml in Hanks' minimal essential medium (MEM) containing 2% bovine serum albumin. Samples of 50 μl were incubated with 5 μl antiserum of the appropriate specificity for 30 min at 4°C . The cells were washed twice with the medium, resuspended in 50 μl of fresh medium, and incubated with 5 μl of FITC-conjugated goat anti rabbit IgG (14 mg ml⁻¹) for another 30 min at 4°C . At the end of the incubation period, the cells were washed three times with the medium and examined in a Leitz Ortholux microscope equipped with a HBO 200-W lamp. Ploem-type vertical illuminator and immersion objectives were used. In each experiment 200 cells were scored for cell surface fluorescence.

*To 10^8 spleen lymphocytes 0.3 ml of an anti-Thy1.2 antiserum was added and incubation was allowed to proceed for 30 min at 4°C . The cells were washed twice in Hanks' MEM and resuspended in 5 ml of a 1/10-dilution of fresh rabbit serum absorbed on agarose. The medium also contained DNase (10 μg ml⁻¹). Incubation was at 37°C for 30 min. Cell debris was removed by extensive washing of the cells in Hanks' MEM, and the incubation with antibodies was carried out as described above.

Table 2 Effect of antibodies and antibody (Fab')₂ fragments of various specificity on the binding of aggregated IgG to murine spleen cells

Antibody specificity*	Pretreatment of cells with IgG (Fab') ₂ % Stained cells	
NRS	42 ± 4	41 ± 5
RBP	44 ± 6	41 ± 6
65,000	6 ± 1	8 ± 2
18-15,000	8 ± 2	7 ± 1
β ₂ μ	7 ± 3	41 ± 7
H-2K ^k	5 ± 2	6 ± 2
H-2K ^k (abs.)	7 ± 2	6 ± 3
Ia ^k	11 ± 3	8 ± 2

Spleen cells from C₃H/He mice were suspended in Hanks' MEM containing 2% bovine serum albumin at a final concentration of 5×10^7 cells per ml. Samples of 50 μl were incubated with 5 μl of the appropriate IgG on (Fab')₂ fraction (15 mg ml⁻¹). After incubation for 30 min at 4 °C, the cells were washed twice in Hanks' MEM. The cell concentration was again adjusted to 5×10^7 cells per ml and 50 μl of FITC-labelled aggregated human IgG (about 1 mg ml⁻¹ in phosphate-buffered saline containing 2% bovine serum albumin and 2 mM sodium azide) was added. After incubation for 30 min at 4 °C, the cells were washed twice and 200 cells were regularly scored for label. The data are the mean ± s.e.m. of three experiments.

* IgG was isolated from immune sera by a combination of sodium sulphate precipitation and Sephadex G-200 chromatography. (Fab')₂ fragments were prepared from the purified IgG by pepsin digestion. (Fab')₂ fragments were freed from remaining intact antibodies by gel chromatography on columns of Sephadex G-200. NRS denotes gamma globulin from a normal rabbit serum. RBP is a human serum protein. The anti-β₂-microglobulin (β₂μ) antiserum was raised in a rabbit against the human protein. This antiserum cross reacts with the mouse homologue. The anti-H-2K^k was raised in (BALB/c × C57BL/6) F₁ mice against A/Sn spleen cells. Absorption of this antiserum was accomplished with C₃H/He thymocytes. Before absorption the antiserum could kill more than 85% of spleen cells and thymocytes from the appropriate strains. After final absorption the antiserum was not cytotoxic against either cell type.

on the cell surface or indeed identical, antibody-induced redistribution and pinocytosis of the Fc receptor would simultaneously eliminate the Ia antigens from the cell membrane. Table 3 shows that although the Fc receptor could be capped away efficiently, no apparent redistribution of the Ia antigens could be demonstrated by the cytotoxicity technique. In a second set of experiments Ia antigens and the Fc receptor were solubilised from a crude C₃H/He spleen cell membrane fraction. The inhibition of anti-Ia^k induced cytotoxicity measured separately on spleen cells from BALB/c and C₃H/He mice was estimated before and after the Fc receptor had been removed from the other solubilised membrane proteins on an aggregated IgG column. Although more than 80% of the Fc receptor could be eliminated from the bulk of material passing unretarded through the column, the content of Ia antigens was unchanged in this fraction. Furthermore, the isolated Fc receptor did not measurably impede the cytotoxic activity of the anti-Ia^k serum.

Trypsin treatment of spleen cells does not affect the presence of the Fc receptor on B cells³. Accordingly, C₃H/He spleen cells were digested with trypsin as described³ and the amounts of Fc receptor and Ia antigens reacting with the appropriate antisera on the cell surface were quantitatively estimated before and after the proteolytic digestion. Whereas trypsin treatment did not change significantly the amount of antibodies bound to the Fc receptor, more than 80% of the Ia antigens were lost. Thus, our results fail to demonstrate a physical association between the Fc receptor and the Ia antigens.

In conclusion, murine B cells seem to carry a glycoprotein which binds to antigen-antibody complexes. Antiserum against this macromolecule abolishes the binding of aggregated IgG to B lymphocytes, demonstrating that the isolated polypeptide chain constitutes the whole or part of the Fc receptor. Although antibodies against Ia also impede the binding of aggregated IgG to B cells, several attempts to demonstrate the presence of

Ia antigenic determinants on the Fc receptor were unsuccessful. This information, together with the fact that the molecular weight of the Ia antigens is only about half that of the isolated Fc receptor¹⁰⁻¹³, suggests that the Fc receptor is distinct from the Ia antigens. The effect of antisera against Ia antigens to displace aggregated IgG does not seem to depend on a physical linkage between these antigens and the Fc receptor but may merely reflect a situation where the Fc receptor in the cell membrane is located adjacent to the Ia antigens.

Table 3 Relationship between Ia antigens and the Fc receptor on the cell surface

Antibody specificity*	Target cells	Pretreatment of cells	Dead cells (%)
18-15,000	H-2 ^k	NRS	47 ± 4
18-15,000	H-2 ^s	NRS	44 ± 3
Ia ^k	H-2 ^k	NRS	55 ± 9
Ia ^k	H-2 ^s	NRS	6 ± 3
Ia ^s	H-2 ^k	NRS	4 ± 2
Ia ^s	H-2 ^s	NRS	41 ± 6
18-15,000	H-2 ^k	18-15,000	6 ± 4
18-15,000	H-2 ^s	18-15,000	8 ± 3
Ia ^k	H-2 ^k	18-15,000	51 ± 7
Ia ^s	H-2 ^s	18-15,000	44 ± 5

Spleen cells from C₃H/He (H-2^k) or A.SW(H-2^s) mice were suspended in Eagle's MEM with 10% FCS at a final concentration of 20×10^6 cell per ml. Samples of 200 μl were pretreated with 50 μl of antiserum against the Fc receptor or with normal rabbit serum (NRS) for 4 h in a 5% CO₂ atmosphere at 37 °C. After incubation the cells were washed twice in cold Hanks' MEM with 5% FCS. The cell concentration was adjusted to 1×10^6 cells per ml, 25 μl samples were subjected to the cytotoxicity assay with use of the antiserum indicated. Dead cells were stained with Trypan blue. At least 200 cells were examined in each test. The data are the mean ± s.e.m. of three to five experiments.

* The A.TH anti-A.TL (anti-Ia^k) and the A.TL anti-A.TH (anti-Ia^s) antisera were given by Dr G. Hämmerling. All antisera were heat inactivated before use and freed from aggregated IgG by ultracentrifugation.

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Tumour rejection properties of solubilised TSTA from an SV40-induced neoplasm

THE neoplasm mKSA, an SV40-induced sarcoma in BALB/c strain mice has strong, SV40-specific tumour rejection antigens (TSTA). This parental line is also very oncogenic with a tumour dose 50 (TD₅₀) of 10³ cells. A subline, passaged in monolayer tissue culture (mKSA-TC), has a much reduced tumour-inducing capacity (TD₅₀ = 2 × 10⁶–2 × 10⁷ cells). Of these mKSA-TC cells 1 × 10⁵ will immunise syngeneic hosts against a challenge of 1 × 10⁶ mKSA cells carried in an ascitic form (mKSA-ASC); immunisation against other SV40-induced BALB/c sarcomas (for example, SVA31C14 and E4) has also been achieved, but not against Adj-PC-5 (ref. 1) nor against Meth A (ref. 2), both neoplasms carrying specific TSTAs and syngeneic with BALB/c but induced by chemical carcinogens.

Recent work in our laboratory showed that methods developed for isolation and solubilisation of biologically active H-2, histocompatibility antigens^{3,4} could be adapted to obtain soluble immunogenic TSTA from both RNA and DNA virus-transformed neoplasms^{5–7}. Preliminary results were reported of solubilised TSTA with tumour rejecting activity from the non-virus-producing tumour, mKSA, induced by SV40⁸. We now report biological activity of ten consecutive solubilised preparations prepared from membranes of either the mKSA-TC or mKSA-ASC forms of this neoplasm.

The antigen preparations assayed for tumour rejection are designated CM, crude membrane; CS, crude soluble; F-2 and F-3 fractions prepared from Sephadex G-150 chromatography (the included volumes). Tumour cells (2–6 × 10⁶), either from the ascites form or from the cultured subline were collected in Tris-buffered saline, pH 7.4. Cells were washed twice in a solution containing 0.145 M NaCl, 0.005 M Tris-buffer, pH 7.4 and 2 × 10^{–4} M MgSO₄; this solution was adjusted with distilled water to 0.125 M NaCl. The cells were equilibrated in a

cell disruption bomb at 400 pounds inch^{–2} N₂ for 30 min and released rapidly to effect disruption of 90% or more of cells. After removal of nuclei and cell debris the supernatant was centrifuged at 55,000g for 90 min, the new supernatant was discarded and the sediment was suspended in 0.05 M Tris, pH 8.4, at approximately 10 mg dry weight per ml. This CM, was digested with papain at a ratio of 1 mg papain to 100 mg protein, at 37 °C in the presence of 0.01 M dithiothreitol; the reaction was stopped after 1 h by adding iodoacetamide to a final concentration of 0.022 M and insoluble sediment removed by centrifugation at 105,000g for 1.5 h. The soluble supernatant was dialysed exhaustively against 0.15 M NaCl–0.01 M Tris, pH 8.2, and then concentrated by ultrafiltration (Amicon, UM2 membranes). The resulting CS material was applied to Sephadex G-150, the eluate collected and a chromatogram obtained by measuring its absorbance at 280 nm. Fractions F-2 and F-3 were collected and used in the *in vivo* assays. Protein concentrations of CM were determined by dry weight and of CS and the F-2 and F-3 fractions by the method of Lowry *et al.*⁸.

As Table 1 shows, all 10 antigen preparations constituting CM, CS or the chromatographed F-2 and F-3 fractions were effective in immunising BALB/c mice against challenge with the syngeneic mKSA sarcoma. mKSA(TC) and mKSA(ASC) were equally effective and thus share the same TSTA in spite of long-continued passage *in vitro* and *in vivo*. Protection was afforded at cell-challenge concentrations far above the TD₅₀ of 10³ cells. The degree of protection was in the range of that provided by a single subcutaneous injection of 10⁶ or 10⁸ mKSA-TC cells; the immunisation afforded was also rapid and complete. Injections prepared in Freund's complete adjuvant (FCA) were not more immunogenic than those prepared in Tris-buffered saline. A precise dose-response relationship was not observed in those experiments (6a, 6b, 7) in which graded doses of antigen were used for immunisation and graded challenges of mKSA cells were used. This may mean that we were immunising with concentrations of antigen above optimal levels. *In vitro* specific lymphocyte stimulation was also

Table 1 Tumour rejection by membrane antigen preparations of sarcoma mKSA

Preparation no.	Type of preparation (source of cells)	Immunisation schedule*	No. of mKSA cells in challenge	No. of + No. inoculated	(Controls)
1a	CM(ASC)	70 µg × 2	10 ⁴ and 10 ⁵ †	0/10	(8/10)
1b	CS (ASC)	100 µg × 2		2/10	(8/10)
2	CM(TC)	1,000 µg × 2		0/10	(8/10)
3	F-2 fraction (TC)	60 µg × 2		1/5	(4/10)
4a	F-2 fraction (TC)	25 µg × 2	” ”	4/10	(10/10)
4b	F-3 fraction (TC)	100 µg × 2	” ”	2/10	(10/10)
		50 µg × 2			
		150 µg × 2			
5	CM(ASC)	1,000 µg × 2	” ”	1/9	(9/10)
6a	CM(ASC)	1,000 µg × 2	10 ³ , 10 ⁴ , 5 × 10 ⁵	0/15	(10/15)
		300 µg × 2		0/15	
		100 µg × 2		0/15	
6b	CS (ASC)‡§	100 µg × 2		10 ³ , 10 ⁴ , 5 × 10 ⁵	
		30 µg × 2	0/15		
		10 µg × 2	0/15		
		10 µg × 2	2/10		
7	CS (TC)	100 µg × 2	7 × 10 ³ , 7 × 10 ⁴	4/10	11/40 (9/10)
		50 µg × 2		3/10	
		25 µg × 2		2/10	
		2.5 µg × 2			
8	CS (ASC)	See text for results of Winn assay			
9	CS (TC)¶	50 µg × 2	5 × 10 ⁴ , 1 × 10 ⁴	0/8	(14/16)
10	CS (ASC)	30 µg × 2	5 × 10 ⁴	0/5	(6/10)
		10 µg × 2		3/5	

P values for differences between experimental and control groups vary from 0.05 to <0.001

*BALB/c female mice, 8–12 weeks old, were given subcutaneous injections of antigen, twice at 2-week intervals. In experiments 1 to 5, injections were prepared in an equal volume of CFA; others were prepared in Tris-buffered saline (TBS). Controls received CFA or TBS as appropriate.

†TD₅₀ of mKSA (ASC) challenge was 10³ cells.

‡Specific lymphocyte stimulation (LS) was demonstrated in a microculture LS assay using the papain-solubilised CS preparations 6b and 7 (ref. 9).

§TSTA activity was reflected in growth rates as well as in frequency of takes. For example mean tumour volume of BALB/c mice immunised with preparation 7 (2.5 µg × 2), 40 d after challenge was 985 mm³ and 3,756 mm³ for controls.

¶Ratio of 1 mg protein to 50 mg protein used for this preparation.

Table 2 Specificity of immune rejection of mKSA sarcoma in syngeneic BALB/c mice

Treatment immunisation	No. with tumour Total no. (r ²)*	Days to death	Challenge tumours
1a = BALB/c spleen CS†	8/8 (29)	42	mKSA 5 × 10 ⁴ cells
1b = none	7/8 (30.4)	41	mKSA 5 × 10 ⁴ cells
2a = mKSA CS (preparation 10)	0/5† (—)	—	mKSA 5 × 10 ⁴ cells
2b = none	6/10 (60.2)	39	mKSA 5 × 10 ⁴ cells
2a = mKSA CS (preparation 10)	10/15 (25)	50	Meth A 5 × 10 ⁴ cells
2c = none	4/5 (33)	50	Meth A 5 × 10 ⁴ cells

*r², Radius² of subcutaneously growing tumours in mm at 30 d after transplantation.

†CS, papain solubilised membranes; 50 µg injected in CFA × 2, 2 weeks apart. Tumour challenge 2 weeks after last immunisation. Controls received CFA only.

‡These mice were rechallenged after 60 d with 1 × 10⁶ mKSA sarcoma cells and remain negative 45 d after challenge.

observed, using preparations 6b and 7, with concentrations of antigen as low as 0.1 µg per well (0.5 µg ml⁻¹)⁹. This assay presumably reflects a cell mediated immune response.

Our soluble F-2 fraction containing immunogenic material chromatographed as we previously observed with H-2 alloantigens derived from lymphoid cells^{6,7}; nonetheless we also observed our F-3 fraction to be immunogenic. This may represent fragments of the TSTA of low molecular weight produced by excessive papain degradation. Further steps in purification of the mKSA TSTA now in progress, however, should clarify this problem.

In addition to the activity of soluble TSTA from mKSA tumour cells in *in vivo* tumour rejection and in *in vitro* lymphocyte stimulation, specific tumour cell neutralisation was observed using the Winn assay. Sensitised spleen cells from BALB/c mice immunised with several concentrations of the (CS) mKSA could neutralise mKSA tumour cells (Fig. 1). Previous work had shown that the host can recognise TSTA on intact mKSA tumour cells and generate effector cells that specifically destroy the tumour¹⁰.

Specificity of immunogenicity was observed in BALB/c

mice made strongly immune to mKSA antigen and challenged with the syngeneic plasma cell tumour Adj-PC5 (ref. 5). Further demonstrations of specificity are shown in Table 2. Papain-solubilised membranes of BALB/c spleen cells did not confer the capacity to reject mKSA tumour nor did the CS preparation of mKSA sarcoma cells immunise against another BALB/c neoplasm, Meth A, that has its own strong TSTA.

Preliminary comparative studies of the properties of TSTA and H-2 have shown that neither antigen bound significantly to a DEAE-ion exchange column in the presence of 0.05 M NaCl at pH 7.2. In contrast, H-2 bound strongly to a con A affinity column, but TSTA did not (O. H., unpublished). The latter observation suggests that TSTA is a different glycoprotein from H-2, but further purification is required before the antigen can be characterised chemically.

These studies demonstrate that the immunogenic capacity of TSTA like H-2 histocompatibility antigens is preserved by papain digestion and by the limited purification procedures described here. Studies of further purification of SV40-specific TSTA are in progress.

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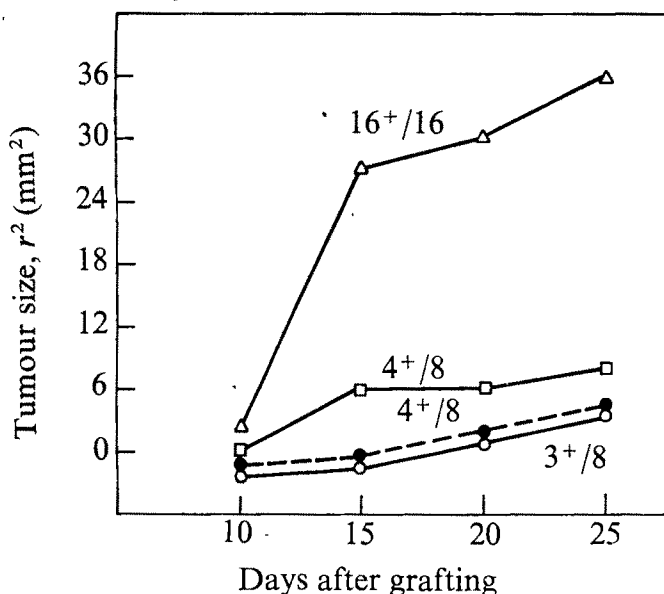


Fig. 1 Results of Winn assay (neutralisation). BALB/c mice were immunised with a single subcutaneous inoculation of preparation 8, CS material in TBS at concentrations of 30 µg (□), 250 µg (○) or 500 µg (●). Two weeks later spleen cells from these immunised groups and from uninoculated controls (Δ) were mixed in a ratio of 100:1 mKSA (ASC) tumour cells and inoculated into BALB/c 8–12-week-old female mice. Sensitised spleen cells from the 250 µg (○) immunised group when mixed with Adj-PC5 (BALB/c) tumour cells and inoculated into syngeneic recipients had no effect on frequency or rate of growth. Data not shown. Differences at 25 d, when 50% of controls had died, in both frequency of progressive growth and mean r² between experimental and control groups were significant ($P < 0.01$).

Possible role for H-Y antigen in the primary determination of sex

In mammals, the genetic basis of sex determination and differentiation seems to be simple^{1,2}. A gene or set of genes on the Y chromosome causes the indifferent embryonic gonad to develop as a testis³. Thus an ovary develops in the absence of the Y and a testis in its presence. The testis then secretes testosterone which induces male development of the accessory glands and ducts^{4,5}. The masculinising action of testosterone on its target cells is mediated by the product of a gene on the X chromosome. This product activates all the genes required for manifestation of the male phenotype in response to circulating testosterone¹. Evidence for this crucial involvement of the

X chromosome in male sexual differentiation comes from a mutation of the relevant gene in the mouse (*Tfm*), resulting in failure to respond to testosterone. A chromosomally XY animal with this mutant gene develops testes, because the Y chromosome is present, but shows no further male differentiation, thus exhibiting the syndrome known as 'testicular feminisation'. This insensitivity to androgen is caused by a mutational deficiency of the nuclear-cytosol androgen-receptor protein not only in mice⁷⁻⁹ but also in man¹⁰. Thus the part that the X chromosome plays in male sexual differentiation has been clarified at the level of an individual gene situated on this chromosome.

The same cannot be said of the part that the Y chromosome plays in inducing testicular development, because no relevant mutation of the presumptive testis-determining gene has been found in any mammalian species, and because we have no clue, therefore, as to the nature of any product specified by this presumptive gene locus. It is clear, however, that the whole Y chromosome is not necessary for this important function. A large part of the mammalian Y chromosome, which as a rule is a relatively minute element, seems to be genetically inert. The observation that an apparent isochromosome of the long arm of the human Y chromosome has no testis-determining function¹¹, indicates that the presumptive testis-determining gene or small cluster of genes resides either on the short arm or on a pericentric region of the acrocentric human Y chromosome.

It is not likely that this presumptive testis-determining gene or set of genes specifies an enzyme or enzymes involved in sex steroid biosynthesis, because the initial biochemical pathways for synthesis of progesterone and oestrogen in the female, and of androgens in the male, are identical. Thus the same enzymes are used by ovary and testis (and to a certain extent by the adrenal) in the production of both male and female sex hormones. Moreover, development of the indifferent gonad into a testis under the influence of the Y chromosome begins as soon as the migration of primordial germ cells from the yolk sac to the gonadal ridge is complete, at which stage endocrine organs in general are not yet fully developed. Thus a cell surface protein or set of proteins concerned in cell-cell recognition and interaction might seem a likely candidate for the gene product or products responsible for initiating testicular differentiation (see below). Under the influence of the Y chromosome, somatic elements of the primitive gonadal ridge differentiate into interstitial cells and Sertoli cells, and as they do so primordial germ cells and Sertoli cells become enclosed within seminiferous tubules, whereas the interstitial cells remain outside. Interaction between the surfaces of somatic elements and germ cells seems essential for the organisation of testicular structure.

By serological methods, those proteins (or the sugar moieties of glycoproteins) which reside in the plasma membrane and are accessible to antibody can be recognised as the surface antigens of intact viable cells. We propose that one such antigen, H-Y (histocompatibility-Y), may be the direct product of the testis-determining gene postulated above.

Rejection of male skin grafts by females within an inbred strain of mice is due to H-Y antigen, found on the cells of all males, but absent from those of normal females (ref. 12; review in ref. 13). Using antibody raised in female mice against cells grafted from males of the same inbred strain, we have found that the cell surface component recognised as H-Y antigen has been highly conserved in vertebrate evolution^{14,15}. Our study of H-Y antigen expression in vertebrates has revealed that H-Y is not in fact specific for males, but rather for the heterogametic sex. Thus in birds and in certain species of Amphibia, female heterogamety of the ZZ/ZW type prevails, and in these animals it is the female whose cells express H-Y antigen¹⁶.

Widespread evolutionary conservation of a particular cell surface component indicates the invariant persistence of a specific function. Our hypothesis is that in the case of H-Y

antigen, this invariant specific function may be to direct the indifferent embryonic gonad to develop towards whichever mature gonad, testis or ovary, typifies the heterogametic sex of each species (testis in XY males and ovary in ZW females).

In mammals the proposed identity of H-Y antigen and the testis-determining gene product can best be tested on exceptional individuals whose gonadal sex does not coincide with their chromosomal sex. In mice and other rodents, a mutational suppression of the Y-linked testis-determining gene should give rise to ovaries in mutant XY individuals, since XO rodents are fertile females¹⁸. According to our hypothesis, such XY individuals would lack H-Y antigen. In man, a similar mutation should give 'streak gonads' in mutant XY subjects, because XO women are sterile¹⁷. Assuming that their streak gonads began as ovaries, these XY women should also type negative for H-Y antigen. On the other hand, a transfer of the testis-determining gene to an autosome, or alternatively, a mutational acquisition of testis-determining function by a dormant autosomal gene, should give rise to testes in mutant XX individuals. These we predict would be typed positive for H-Y antigen. Indeed our preliminary observations indicate that certain XX human males whose cells we have examined express H-Y antigen. Moreover XX mice that are 'sex-reversed' to males by the autosomal dominant, *Sxr* (ref. 18), are also positive for H-Y antigen (D. Bennett, E. A. B., B. M. Cattenach, B. J. Mathieson, M. Scheid, and K. Yanagisawa, unpublished). The original breeding data and cytological observations indicate that if *Sxr* represents a piece of the Y translocated to an autosome, then that piece is so small as to be undetectable karyotypically¹⁸. Therefore the fact that *Sxr*+/+ males express H-Y antigen clearly shows either that the testis-determining gene and the H-Y locus are extremely closely linked, or that the two are in fact one and the same.

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Expression of H-Y (male) antigen in phenotypically female *Tfm*/Y mice

THE X-linked testicular feminisation mutant (*Tfm*) in the mouse produces chromosomally male animals, *X^{Tfm}/Y*, with small testes but no other internal reproductive organs, and with female external genitalia¹. This condition results from

failure of target tissues to respond to testosterone, so that all characters of maleness that are testosterone-dependent are absent in males²⁻⁴.

In addition to the development of testes, there is one other attribute of maleness in the mouse which seems not to require testosterone; this is the expression of H-Y antigen, a cell surface component found on all cells of male mice, but on no cells of females⁵. Although some data are controversial, and while quantitative effects of testosterone can be envisaged, the bulk of evidence suggests that H-Y is expressed whenever the Y chromosome is present, and not otherwise.

Among the supporting evidence is the following: (1) Expression of H-Y antigen is not affected by the single-X compared with the two-X constitution; that is, XXY male mice have H-Y antigen whereas XO females do not⁶. (2) Testicular teratomas are H-Y⁺ when they have the Y chromosome but not when Y is lost as a consequence of aneuploidy that may occur on passage⁷. (3) Genetic data coupled with skin-grafting experiments suggest not only that H-Y is, determined by the Y chromosome, but that it may exist in different allelic forms in males of different strains⁸, this is, incidentally the best indication so far that the H-Y-determining gene of the Y chromosome is 'structural' rather than 'regulatory'. (4) Male haemopoietic cells colonising lethally irradiated females continue to express H-Y (ref. 9). (5) In spite of the evidence that neonatal castration may reduce the immunogenicity of male skin grafts¹⁰, deprivation of testosterone has not been shown to eliminate H-Y antigen qualitatively; nor did the administration of testosterone induce H-Y in females¹¹. (6) Evidence that newborn female skin that has been resident on males is rejected on regrafting to females, implying acquisition of H-Y in the male environment, is both claimed¹² and disputed¹³. (7) The heterogametic sex, whether male or female, is H-Y⁺ in other vertebrate species¹³; in fact we must now recognise that the term 'male antigen' is a misnomer in reference to H-Y.

The most likely explanation for all these findings is that H-Y is controlled by a gene or genes on the Y chromosome, and that neither testosterone *per se* nor the male environment is required to elicit activity of this gene or genes.

The evidence is that all testosterone-dependent characters are lacking in *Tfm/Y* animals and that even the *Tfm/Y* embryo is unresponsive to testosterone⁴. Therefore the finding that we report here, namely that *Tfm/Y* mice are H-Y⁺, is strong evidence that the expression of H-Y is testosterone independent.

Mice from the Harwell *Tfm* stock are maintained by crossing *Tfm*+/+/+ *Blot*+/+ *Ta*+/+ *Y* and *Tfm*+/+/+ *Ta*+/+ to + + *Blot*/Y in alternate generations. For the tests described below, the *Tfm* animals were *Tfm/Y* from this stock, the control females were their *Tfm* + + / + *Ta* + siblings, and the control males were + + *Blot*/Y siblings.

Anti-H-Y sera were prepared in C57Bl/6 (abbr B6) females by repeated grafting of B6 male skin. The anti-H-Y activity of such sera is demonstrable by the complement-dependent cytotoxicity assay on sperm¹⁴ or dissociated epidermal cells¹⁵. We used 3 types of experiment: (1) The cytotoxicity assay with anti-H-Y serum on epidermal cells from *Tfm/Y* mice (Fig. 1).

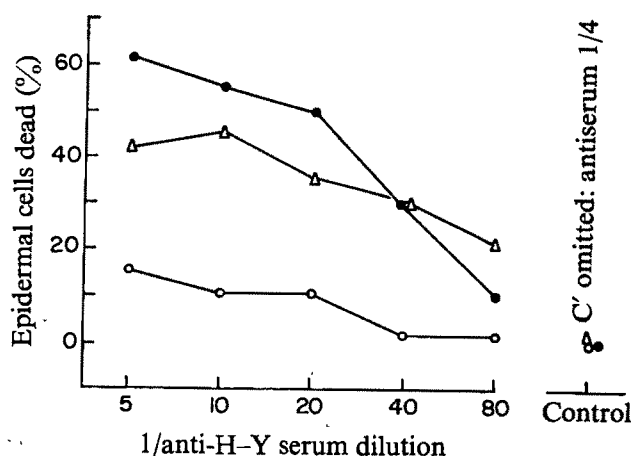


Fig. 1 Demonstration of H-Y antigen on dissociated epidermal cells of *Tfm/Y* mice by the complement-dependent cytotoxicity assay test with anti-H-Y serum. The data in this figure are from a representative test with two mice of each genotype. The values shown are mean readings for the pairs of mice. The anti-H-Y serum was preabsorbed twice with female epidermal cells (4 parts serum undiluted: 1 part packed cells) at 0-4 °C. Complement source was selected rabbit serum, absorbed with thymocytes, lymphocytes, and epidermal cells in the presence of EDTA (see ref. 15), diluted 1/6 before use. Percentage dead epidermal cells (ordinate) calculated by the formula $\{(a-b)/(100-b)\} \times 100$ where *a* is the % dead cell count (Trypan blue stained cells) after incubation with antibody and complement; and *b* is the similar count with antiserum omitted. This somewhat lower reaction of *Tfm/Y* epidermal cells, compared with control male cells is not significant, and was not a feature of other similar tests. Δ, *Tfm/Y*; ●, + + *Blot/Y* (normal male control); ○, *Tfm* + + / + *Ta* + (normal female control).

(2) Absorption of anti-H-Y serum with spleen cells from *Tfm/Y* mice followed by testing for cytotoxicity on sperm (Fig. 2). (3) Grafting of *Tfm/Y* skin to B6 females to determine whether this would presensitise them for a second-set response to subsequent grafting of B6 male skin (Table 1). Figure 1 gives one representative example out of a total of five positive cytotoxicity tests with anti-H-Y serum on *Tfm/Y* epidermal cells. Figure 2 shows two positive absorption tests with *Tfm/Y* spleen cells. Table 1 summarises the results of the grafting experiments, in which *Tfm/Y* skin grafts sensitised B6 females to subsequent B6 male skin grafts. In all of these tests, cells from *Tfm/Y* mice have proved indistinguishable from those of normal males in their expression of H-Y.

We conclude that H-Y is not testosterone dependent. Since the *Tfm/Y* animals have small testes, which seem fairly normal at foetal to neonatal stages⁴, one must consider whether it is the presence of testes which determines the production of H-Y antigen or whether the presence of the Y chromosome itself is sufficient. The concept that some influence of a gonad could affect surface antigenicity of cells of other tissues, seems very difficult to accommodate within existing knowledge. Furthermore, it is not yet certain when Y-antigen expression begins during development, and so it may in fact precede

Table 1 Second-set rejection of B6 male skin by B6 females previously grafted with *Tfm/Y* skin*

First graft (number of B6 females grafted)	Rejection time (d) of B6 male skin grafted 2-3 months later	MST† (confidence limits)
<i>Tfm/Y</i> (15)	11, 12, 13, 13, 13, 14, 14, 14, 15, 15, 16, 16, 17, 17.	14 (12.7-15.4)
+ + <i>Blot/Y</i> ; male controls (13)	12, 12, 15, 15, 16, 16, 16, 17, 17, 18, 18, 22, 22.	15.5 (13.5-17.8)
<i>Tfm</i> + + / + <i>Ta</i> +; female controls (8)	12, 16, 17, 18, 19, 21, 21, 40.	18 (16.4-19.7)

*Summary of data for two experiments involving four *Tfm/Y* graft donors and three donors for each of the two control groups; these donors also provided spleen cells and tail epidermal cells for the serological tests (Figs 1 and 2) Trunk skin grafted according to Billingham and Medawar¹⁷, using Band-Aids instead of plaster casts; rejection times scored visually.

†Median survival time (MST) and confidence limits of the median calculated by the method of Litchfield¹⁸.

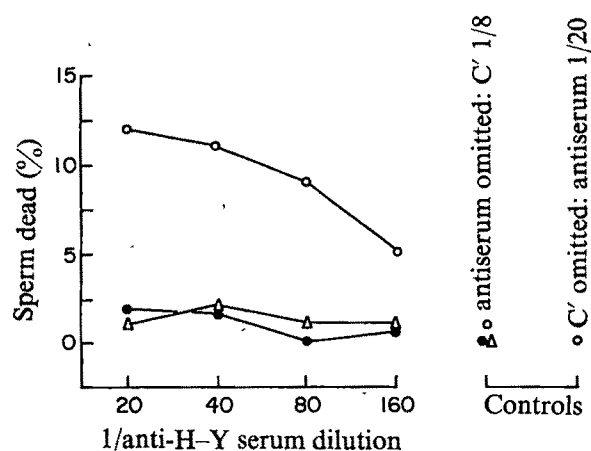


Fig. 2 Demonstration of H-Y antigen on *Tfm/Y* spleen cells by absorption of cytotoxic activity from anti-H-Y serum. The data shown illustrate a representative test. Each point represents the mean value obtained when spleen cells from two different mice of the same genotype were tested separately. The anti H-Y serum (preabsorbed with female epidermal cells, see footnote to Fig. 1) was diluted 1/4 and absorbed in 0.1-ml volumes with spleen cells from *Tfm/Y* mice or control mice as indicated, for 30 min at 0–4 °C; after centrifugation to remove the cells, the absorbed serum was titrated against sperm for residual cytotoxic activity, using horse serum diluted 1/8 as the complement source. The tests were read 'blind' with absorbed antiserum sample coded. Percentage sperm dead (ordinate) was calculated by the formula $\{(a-b)-(100-b)\} \times 100$, where a is number of sperm dead, and b is the % dead sperm in original suspension (method described in detail elsewhere¹⁴). Sperm were obtained from unrelated normal mice. H-Y antiserum absorbed with: △, *Tfm/Y*; ●, + + *Blo/Y* (normal male controls); ○, *Tfm* + + + *Ta*+ (normal female controls).

testis formation. We prefer to consider that the detection of H-Y in these *Tfm/Y* phenotypic females with a Y chromosome constitutes further compelling evidence that H-Y is indeed Y dependent, not male dependent.

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Effect of morphine on a depolarising dopamine response

THE structural complexity of nervous tissues and the presence of non-neuronal elements have hindered investigations into the actions of morphine at the cellular level in the vertebrate central nervous system, and contribute to the difficulty of determining whether morphine acts pre- or postsynaptically. Although presynaptic effects of morphine are documented, to our knowledge there has been no demonstration of an effect

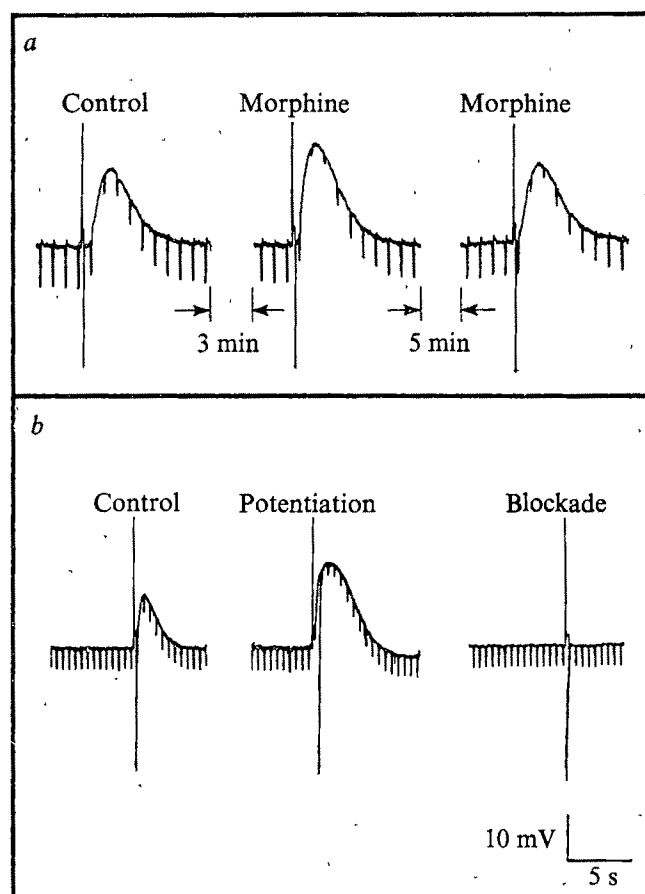


Fig. 1 *a*, Potentiation of the DA response by low morphine concentrations. DA iontophoretic charge, 500 nC. Current pulse amplitude was maintained constant at 0.26 nA for the duration of the experiment. *b*, Potentiation of the DA response by low morphine concentrations followed by blockade of the response at higher morphine concentrations in the same cell. Morphine added to the bath (0.5 μM) caused an increase in the DA response amplitude. An excess of morphine (24 μM) blocked the DA response. Potentiation could be attained with 0.2–0.6 μM morphine and blockade with 8–12 μM morphine. DA iontophoretic charge, 300 nC. Current pulse amplitude was maintained constant at 0.15 nA for the duration of the experiment. Morphine in the concentration range tested (0.1–50 μM) had no effect on the passive membrane properties. Polarising current pulses were passed across the membrane to determine conductance changes. Standard electrophysiological techniques using a bridge circuit recorded membrane potential and passed polarising current intracellularly simultaneously. Glass microelectrodes filled with either potassium acetate or chloride and with resistances greater than 30 MΩ were used for recording. During recording, the 35-mm Falcon culture dish on which the cells were grown remained covered except for a 1-cm hole in the centre to allow for entry of electrodes. The culture dish was maintained at 37 °C and gassed continually with a stream of water-saturated 10%–95% carbon dioxide–air mixture. There was negligible evaporation of growth medium during any one recording session. 3,4-Dihydroxyphenylethylamine (dopamine)-HCl (Sigma) was prepared in aqueous 1 M concentrations (pH 3–4) and used to fill iontophoretic glass microelectrodes, the final resistance of which was adjusted to 10–20 MΩ by bumping the electrode tip.

of morphine on postsynaptic neurotransmitter receptors using intracellular recording techniques.

We have studied the effects of morphine on the dopamine response of the somatic cell hybrid 108CC-15. The cell line is a product of the fusion of cells from the mouse neuroblastoma N18TG2 and the rat glioma C6-BU-1 and expresses a number of neuronal phenotypes¹⁻⁴. It is a homogeneous population of cells, allowing intracellular recording from single cells free of organisational complexity or synaptic input. The biochemical and electrical properties of the cell line have been described in detail elsewhere¹.

When dopamine (DA) is applied iontophoretically, a depolarising response, mediated by a conductance increase, is seen in 64% of the cells. The average resting membrane potential was -42.5 mV with an average input resistance of 39 m Ω . The DA response has a reversal potential of -15.9 mV (± 1.2). Repeated application of DA at close time intervals resulted in desensitisation of the response. The response is blocked by the catecholamine antagonists bulbocapnine, chlorpromazine, and phentolamine; the dopamine metabolite 3-methoxytyramine, and the acetylcholine antagonists hexamethonium and α -bungarotoxin, are without effect. Details of cell growth for this electrophysiological study and additional characteristics of the DA response are published elsewhere²².

Fresh solutions of morphine sulphate (Mallinckrodt) and naloxone HCl (Endo Laboratories) were prepared before each

experiment. After each drug application to the bath, adequate time was allowed (5–10 min) to allow equilibration of the drug in the medium. Morphine was applied iontophoretically from glass micropipettes filled with 1% morphine sulphate. Morphine (0.1 – 50 μ M) and naloxone (1 – 10 μ M) had no effect on the passive membrane properties. Current pulses were injected continuously during drug applications and demonstrated that there were no changes in membrane resistance or membrane potential.

Morphine at low bath concentrations (0.2 – 0.6 μ M) increased the DA response amplitude up to 75% above control levels (Fig. 1a). The increase was transient, since the response amplitude was observed to return to control levels and in most cases, it was not possible to repeat the potentiation on the same cell after the initial exposure to a low morphine concentration. The potentiation was also observed with iontophoretic application of morphine.

Higher bath concentrations of morphine (8 – 12 μ M) blocked the DA response and it remained blocked as long as morphine was present in the bath. Figure 1b shows potentiation of the DA response in a single cell at low concentrations but that on addition of more morphine to the bath, the DA response was blocked. Iontophoretic application of morphine blocked the DA response and this block was dose dependent and reversible after diffusion of the drug into the medium (Fig. 2a). When morphine was added to the bath in sufficient concentration to block the DA response, addition of equimolar naloxone reversed the blockade and the DA response recovered to control levels (Fig. 2b). This observation agrees well with the known antagonist action of naloxone on morphine.

In both peripheral and central nervous tissues, various lines of evidence have suggested both a pre- and postsynaptic effect of morphine. A presynaptic action of morphine is argued from studies of the uptake, metabolism and release of neurotransmitters⁵⁻¹³. Inferences on the postsynaptic action of morphine are based on morphine interaction with neurotransmitter antagonists¹⁴⁻¹⁷. In a separate study, Bradley and Dray¹⁸ found both potentiation and inhibition of neurones when morphine was applied iontophoretically to brain stem. The potentiation was observed to "desensitise". Our results support the view that morphine acts at least postsynaptically. Pert and Snyder¹⁹ first demonstrated that opiate receptors exist in the brain; Klee and Nirenberg²⁰ have demonstrated that 108CC-15 has opiate receptors with characteristics similar to brain receptors. That morphine influences the DA response suggests the existence of some relationship between opiate receptor binding and neurotransmitter receptors. Although Pert and Snyder²¹ have shown that catecholamines do not block binding of morphine to its receptor, their results do not preclude a specific interaction of morphine with the DA receptor.

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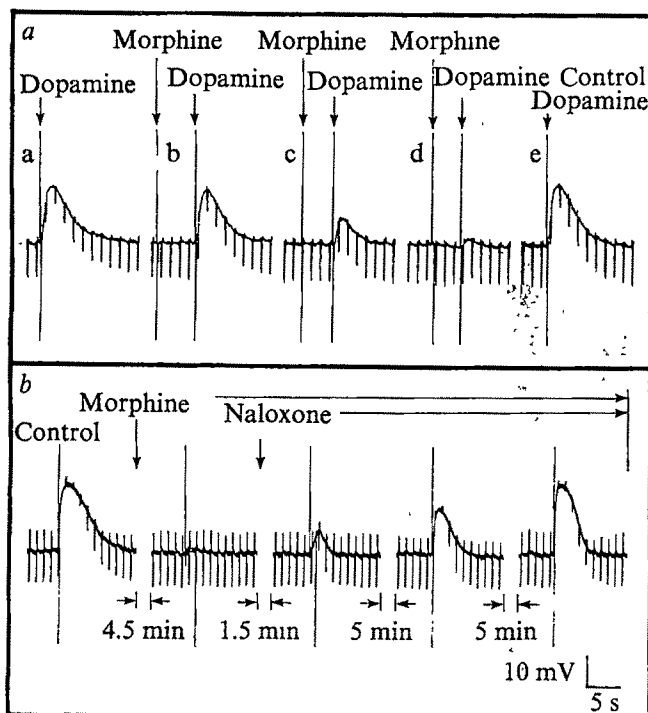


Fig. 2 a, Dose-response characteristic of the morphine blockade. Morphine applied iontophoretically in increasing quantities decreased the DA response amplitude in a dose-dependent fashion. The blockade was reversible after diffusion of the drug into the medium and the blockade could be repeated on the same cell. In recordings b, c, and d the first stimulus artefact represents morphine application and the second represents DA application. The time between drug applications was 2 min. a, 300 nC DA; b, 50 nC morphine, 300 nC DA; c, 100 nC morphine, 300 nC DA; d, 200 nC morphine, 300 nC DA; e, 300 nC DA. Current pulse amplitude was maintained constant at 0.5 nA for the duration of the experiment. b, Reversal of the morphine block by naloxone. After sufficient morphine was added to the bath to block the DA response (7.3 μ M), an equimolar amount of naloxone was added and the DA response returned to control levels. DA iontophoretic charge, 300 nC. Current pulse amplitude was maintained constant at 0.3 nA for the duration of the experiment. Naloxone added to the bath alone had no effect on the membrane passive properties nor the DA response. In similar experiments, longer time periods were allowed after the addition of morphine to the bath to ensure equilibration and that return of the DA response after addition of naloxone to the bath was not purely coincidental.

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Receptor potential and impulse initiation in two varieties of reptilian muscle spindle

AMONG muscle spindles, those in snake and lizard are the simplest in structure, consisting of a single intrafusal muscle fibre innervated by a single sensory and several motor nerve fibres. Two varieties of spindle occur: one type, the long-capsule spindle has a longer and more slender capsule than the other, the short-capsule spindle¹. They differ in their responses to stretch; the response of the long-capsule spindle depends mainly on degree of stretch while the short-capsule spindle responds both to velocity and amplitude of stretch^{2–4}.

Sensory transduction in muscle spindles involves the following steps: transmission of mechanical stimulus (stretch) through intrafusal fibres, generation of receptor potential in the sensory terminals and impulse initiation in the afferent fibre. While there is information on the mechanical transmission in reptilian spindles⁵ and on the characteristics of the impulse initiating process⁶, there has been no study of the receptor potential in long- and short-capsule reptilian spindles in response to stretch. We report here some features of receptor potential characteristic of each type of snake spindle which may account for differences in the early adaptation of impulse discharge between the two types of spindle.

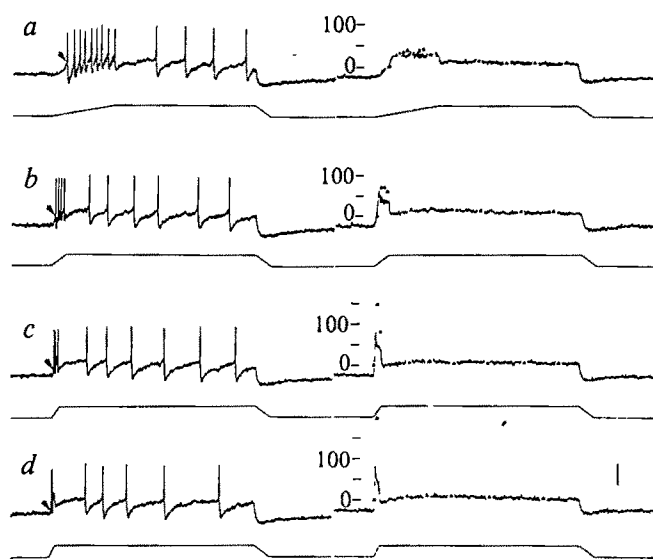
Muscle spindles in ventral costocutaneous muscles of garter snake (*Thamnophis*) were used. The technique for dissection of the muscle and the ionic composition of the bathing solution have been described elsewhere⁷. After visual identification of the type of spindle under the dissecting microscope and dark field illumination, a single spindle was isolated with some extrafusal fibres which served as a support. Each end of the muscle was tied to a nylon rod which was connected to one of a pair of Brush pen motors. The muscle spindle was stretched to both sides by a computer (LINK) controlled activation of Brush pen motors at a rate of once every 5 s. The isolated spindle was brought near the interface between snake Ringer's solution and liquid paraffin and the nerve was lifted into liquid paraffin on one of a pair of glass pipettes filled with agar-Ringer. The other pipette was placed in the Ringer's solution. Each pipette was connected to one of a pair of half calomel cells and signals from the nerve were amplified by a PAR amplifier. Most of the experiments were done at the muscle length at which slack was just taken up. Tetrodotoxin (TTX) of 10⁻⁷–10⁻⁸% was used to block impulse initiation in the ending. After each experiment the nerve was crushed with a pair of fine forceps at the region close to the capsule. In all cases examined no noticeable movement artefact was observed. Experiments were performed at room temperature (about 25 °C).

Figures 1 and 2 illustrate examples of responses of a short-capsule and a long-capsule spindle, respectively, to ramp-and-hold stretch of increasing velocity (from a to d) before (left column) and after (right column) application of TTX. Several components may be recognised in the dynamic component of the receptor potential particularly in short-capsule spindles. At the beginning of stretch there is a steep rise of receptor potential (initial component) and at the end of the ramp the potential swings back to a lower level (dynamic-static fall)

often making an undershoot (dynamic-static undershoot). Between the initial component and the dynamic-static fall the potential shows either a plateau or gradual decay or increase depending upon the type of spindle and the velocity and amplitude of stretch. During the hold phase of stretch the potential gradually declines (adaptive static fall) and at the end of stretch the potential may show "release undershoot" before returning to the original level (the above terminology adopted from ref. 8). In the long-capsule spindle the initial component and the dynamic-static undershoot are both usually lacking. The initial component may correspond to the initial burst of discharge which often occurs in short-capsule, quite rarely in long-capsule spindles. The dynamic-static undershoot may correspond to the pause of impulse activity which occurs just after the ramp as may be seen in Fig. 1. The above features of receptor potentials characteristic of the short-capsule and the long-capsule spindles are strikingly similar to those recorded from the primary and the secondary endings, respectively, in cat muscle spindles⁸. In order to examine the relation between dynamic components of receptor potential and impulse discharge the threshold depolarisation of receptor potential (zero frequency on ordinate) for initiating a train of impulses was determined by extrapolating the approximately linear relation between mean static frequency and receptor potential 0.5 s after the ramp phase of stretch of varying amplitude. The scaling of frequency on the ordinate was then adjusted so that the mean static frequency and the receptor potential 0.5 s after the ramp coincide each other. The results thus obtained are shown in Figs 1 and 2 as filled triangles superimposed on each receptor potential. In both types of spindle when the velocity of stretch is slow the frequency of impulse discharge follows relatively well the dynamic component of receptor potential. As the velocity of stretch increases the discrepancy between the two parameters increases progressively. Particularly in short-capsule spindles the dynamic impulse frequency rises far above the level of receptor potential.

The progressive discrepancy between discharge frequency and the receptor potential with the increase in velocity of stretch could be accounted for by accommodative process occurring

Fig. 1 Responses of a short-capsule spindle to ramp-and-hold stretch of increasing velocity (from a to d, see each lower trace) before (left column) and after (right column) application of 10⁻⁸ TTX. Records were all reproduced from tape. A pair of records in a single row are responses to an identical stretch. Velocity of stretch (mm s⁻¹); a, 1.0; b, 5.0; c, 10.0; d, 15.0. Amplitude of stretch, 500 µm. Arrows indicate threshold level of receptor potential for the first spike. Triangles, frequency of impulse discharge before application of TTX (explanation of scaling in text). Ordinate, impulses s⁻¹. Time calibration, 0.5 s. Voltage calibration (250 µV) applies to all records of response.



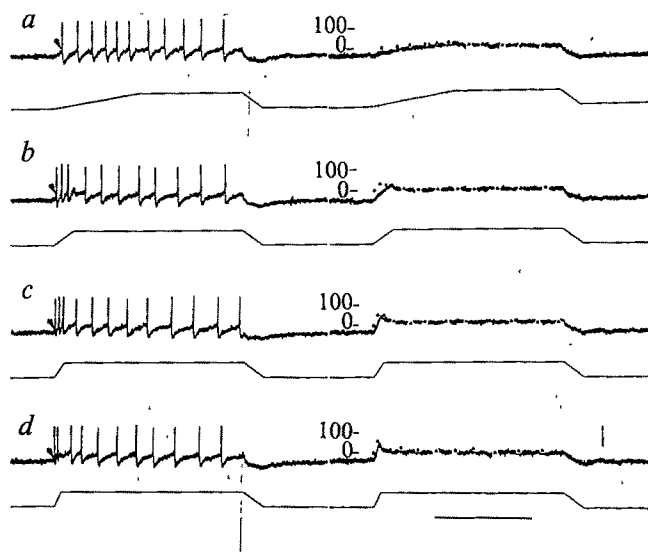


Fig. 2 Responses of a long-capsule spindle to ramp-and-hold stretch of increasing velocity (from *a* to *d*) before (left column) and after (right column) application of 10^{-6} TTX. Records are arranged in the same way as in Fig. 1. Velocity of stretch (mm s^{-1}); *a*, 1.0; *b*, 5.0; *c*, 10.0; *d*, 15.0. Amplitude of stretch, 350 μm . Arrows and triangles, threshold depolarisation for the first spike and impulse frequency plotted as in Fig. 1 respectively. Ordinate, impulses s^{-1} . Time calibration, 0.5 s. Voltage calibration (200 μV) applies to all records of response.

in the spike initiating site. In fact, with decrease in stretch velocity substantial increase in the threshold depolarisation for the first spike may be seen in Figs 1 and 2 (arrows). On the basis of accommodation it may be predicted that the larger the dynamic component of receptor potential the larger the discrepancy would occur between impulse frequency and receptor potential as illustrated in Figs 1 and 2. Another possibility for the discrepancy may be the preferential suppression of the dynamic component of receptor potential by TTX. This seems less probable because (1) after application of TTX the dynamic discharge is always the last to be blocked, (2) higher concentration of TTX (10^{-5}) does not seem to affect appreciably the receptor potential and (3) as in Figs 1 and 2 the dynamic impulse frequency and the dynamic receptor potential at low velocity of stretch coincide rather well with each other. Whatever the underlying mechanism is, however, it may be concluded that the basis for the difference in stretch-velocity sensitivity between the two types of spindle is due mainly to the difference in the dynamic component of the receptor potential. Taking the previous findings^{5,6} into consideration it follows that either the mechanical event occurring at the ultrastructural level particularly in the sensory ending-intrafusal fibre contact region and/or in the ionic mechanisms for generating receptor potentials in the sensory ending may be responsible for the stretch-velocity sensitivity of snake muscle spindles.

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Binding of aldosterone by mitochondria-rich cells of the toad urinary bladder

ALDOSTERONE causes a large increase in the active transport of sodium by the urinary bladder¹ of the toad. This response, analogous to the effects of the steroid on the kidney, has been the object of considerable biochemical study². Aldosterone also enhances the stimulation by neurohypophyseal hormones of sodium and hydro-osmotic flux^{3,4}. Because the response in sodium transport seems quantitatively related to the increment of cyclic AMP generated by vasopressin⁵, augmentation of the sodium response is presumably related to the larger increment of intracellular nucleotide induced by a given dose of vasopressin in steroid-treated bladders⁶. Using a technique for separating the two major cell types of the bladder mucosal epithelium—the granular (G) and the mitochondria-rich (MR) cells—we found that a response to neurohypophyseal hormones, as reflected by hormone-induced increases in the intracellular cyclic AMP content, is apparently limited to the MR cell⁷. This apparent synergism of the neurohypophyseal and steroid hormones on transport suggests that their loci of action are in close proximity. We therefore examined the binding of ³H-aldosterone by the two major cell types.

To measure the specific binding of ³H-aldosterone by mineralocorticoid receptors, we incubated paired sets of 6–10 intact hemibladders in separate baths. One bath (³H-aldo) contained ³H-aldosterone alone in various concentrations, while the paired bath (control) contained the same concentrations of ³H-aldosterone plus a 1,000-fold excess of unlabelled steroid. After incubation for 90 min, the mucosal cells were removed by immersing the bladders in EDTA–Ringer solution. The MR and G cells of each set of bladders were separated by density gradient centrifugation⁷ and the ammonium-sulphate precipitable ³H-aldosterone was measured in each of the four preparations. The difference in the ³H-aldo and control values for each cell type was assumed to represent specifically-bound or displaceable steroid (Table 1).

We found that the amount of displaceable ³H-aldosterone in MR cell preparations was related directly to the concentration of labelled steroid in the bath. With the exception of a single experiment, there was no significant binding of displaceable aldosterone by G-cell preparations. We also measured the specific binding of corticosterone, the natural glucocorticoid of *Bufo marinus*, by the two cell types. ³H-corticosterone (New England Nuclear, 84 Ci mmol⁻¹), 10^{-8} M, and a 1,000-fold excess of unlabelled corticosterone, were used as in the aldosterone binding studies. The amount of displaceable corticosterone bound by the G cells (2.6×10^{-14} mol per mg protein) was equivalent to that bound by the MR cells (2.95).

The displaceable aldosterone bound by the MR cells was considerably greater in April than values measured during September to March. This increased level of binding persisted until late August when successive experiments at the level of 5×10^{-9} M ³H-aldosterone showed a progressive decrease in the amount of steroid bound by the MR cell (from the expected 28.0) to 9.5, 8.9, 2.9 and 2.1×10^{-14} mol ³H-aldosterone per mg protein. This compared with a value of 2.15 obtained the previous March (Table 1). This marked variability in the amount of aldosterone bound by the MR cells corresponds and may be related to the well known seasonal variations in the responsiveness of the toad bladder transport mechanisms to mineralocorticoid hormones.

The mineralocorticoid/glucocorticoid selectivity of the receptor binding aldosterone was examined by measuring the efficacy with which deoxycorticosterone (DOCA) and cortisol displaced ³H-aldosterone. Hemibladders were incubated in three sets. In addition to ³H-aldo and control sets used in previous experiments, the third set was exposed to either DOCA or cortisol in 200-fold excess as well as ³H-aldosterone.

Table 1 Specifically bound ^3H -aldosterone in separated mitochondria-rich and granular cells

	^3H -aldosterone ($\text{M} \times 10^{-9}$)	Mitochondria-rich cells			Granular cells		
		^3H -aldo	Control	Net	^3H -aldo	Control	Net
November–March	1.2	1.38	0.84	0.54	0.73	0.74	0
	2.0	1.32	0.12	1.20	0.16	0.12	0.04
	2.2	2.59	1.79	0.80	1.23	0.94	0.29
	2.8	3.09	1.68	1.41	0.65	0.48	0.17
	5.0	3.05	0.89	2.15	1.18	0.87	0.30
	20.0	8.50	4.16	4.34	4.15	5.57	0
April–June	1.0	8.70	0.07	8.6	1.50	1.86	0
	4.0	24.00	0	24.0	4.70	6.50	0
	10.0	68.80	0	68.0	18.20	1.68	16.5
	40.0	88.00	1.80	86.2	1.77	1.91	0

Toads (*B. marinus*) of Colombian origin (Tarpon Springs Inc.) were kept in 0.6% saline for at least 3 d to suppress endogenous aldosterone. Cells were derived from two sets of 8–12 urinary hemibladders incubated for 90 min in Ringer containing NaCl, 85 mM; KCl, 4 mM; NaHCO_3 , 17.5 mM; KH_2PO_4 , 0.8 mM; MgSO_4 , 0.8 mM; CaCl_2 , 1.5 mM; dextrose, 10 mM; and ^3H -aldosterone (New England Nuclear, 91.6 Ci mmol $^{-1}$), in the indicated concentrations. The control bath also contained a 1,000-fold excess of unlabelled aldosterone. The mucosal cells were removed by incubation in EDTA–Ringer and the MR and G cells separated by centrifugation on a discontinuous density gradient of Ficoll⁷. The MR and G cells from the ^3H -aldo and control tissues were sonicated and the radioactivity measured in the ammonium sulphate (50% saturated) precipitable fraction of the 110,000g supernatant. The specifically bound steroid in each experiment, the difference between the ^3H -aldo and control, is expressed as mol $\times 10^{-14}$ per mg protein.

As Table 2 shows, DOCA, a potent mineralocorticoid, completely displaced ^3H -aldosterone from the MR cells. Cortisol, primarily a glucocorticoid, displaced only about 24% of the specifically-bound aldosterone, indicating high mineralocorticoid specificity for the ^3H -aldosterone binding sites.

The subcellular distribution of displaceable ^3H -aldosterone was determined in unseparated mucosal cells. Intact hemibladders were incubated as 10 paired sets of ^3H -aldo and control tissues. Both bathing solutions contained 10^{-8} M ^3H -aldosterone and the control bath also contained 10^{-5} M unlabelled aldosterone. After incubation for 90 min the mucosal cells were removed and the two suspensions of mucosal cells were disrupted by pressure homogenisation, which leaves the nuclei intact. The homogenates were fractionated by differential centrifugation and ammonium sulphate precipitation. In bladders incubated in ^3H -aldosterone alone, 1,222 c.p.m. representing approximately 12% of the steroid in the homogenate, sedimented with the nuclei (Table 3). Receptor-dependent uptake of aldosterone by the nucleus is demonstrated by the large reduction in the amount of labelled steroid sedimenting with the nuclei prepared from control tissues (4.6%). The ^3H -aldosterone bound by the ammonium sulphate precipitate prepared from the supernatant fraction of the cell also demonstrated significant displacement by unlabelled steroid. These data, which confirm earlier studies⁸ of subcellular localisation of aldosterone, indicate that the steroid is bound by specific receptors in both the nucleus and the cytoplasm of the mucosal cell.

The identification of the MR cell as the aldosterone-binding locus in the bladder mucosal epithelium is reinforced when

displaceable steroid is determined in the nuclei from isolated MR cells. Using incubation procedures described earlier, the MR cell nuclei from ^3H -aldo (10^{-8} M) and control tissues were isolated. The nuclei from the ^3H -aldo set contained 7,650 c.p.m., which when compared with only 60 c.p.m. in the control nuclei, indicates nuclear accumulation of aldosterone by a specific receptor mechanism in the MR cells.

Our data demonstrate the specific, concentration-dependent binding of aldosterone by the MR cells and localisation of the steroid within the nucleus. These results, together with our demonstration that aldosterone induces the synthesis of protein in MR cells¹⁰, imply a transcriptional effect of aldosterone.

Table 3 Subcellular distribution of ^3H -aldosterone in isolated mucosal cells

Subcellular fraction	^3H -aldo	Control
Nucleus	12.0%	4.6%
Postnuclear pellet	10.6	11.8
Supernatant	84.1	87.5
$-(\text{NH}_4)_2\text{SO}_4$ precipitate	8.4	5.1

Paired sets of hemibladders were incubated as in Table 1; the concentration of ^3H -aldosterone was 10^{-8} M. After 1.5 h mucosal cells were removed by incubation in Ringer containing EDTA (2 mM) with no Ca^{2+} or aldosterone present. Mucosal cells were disrupted by pressure homogenisation; the samples were equilibrated for 30 min with nitrogen at 1,350 pounds inch $^{-2}$ before release⁹. Nuclei were sedimented by centrifugation at 600g for 20 min. The 600g supernatant was centrifuged at 110,000g for 30 min (postnuclear pellet) and protein in this supernatant precipitated by ammonium sulphate (50% saturated). Radioactivity was measured by liquid scintillation and the radioactivity in each fraction expressed as the percentage of that in the original homogenate.

Table 2 Effects of DOCA and cortisol on aldosterone binding in toad bladder MR cells

Date	^3H -aldo	Control	Competing steroid DOCA	Cortisol	%Displacement of ^3H -aldo by competitor
8/28	15.7	6.2	5	—	> 100
8/30	14.7	5.8	—	12.3	27
9/10	7.4	4.5	—	6.8	21
9/18	3.6	1.4	1.2	—	> 100

Three sets of eight hemibladders were used: ^3H -aldo, hemibladders incubated in 5×10^{-8} M ^3H -aldosterone; control, hemibladders incubated in 5×10^{-8} M ^3H -aldosterone plus 5×10^{-6} M unlabelled aldosterone; and either DOCA or cortisol, hemibladders incubated in 5×10^{-8} M ^3H -aldosterone plus the competing steroid (10^{-6} M). The values given (in mol $\times 10^{-14}$ ^3H -aldosterone per mg ammonium sulphate-precipitable protein) are the amounts of ^3H -aldosterone bound by the MR cells in each set of hemibladders.

These observations, together with earlier evidence that the MR cell is the target of neurohypophyseal hormones⁷, indicate that the MR cell is the initial locus of action of natriuretic hormones. Other investigators have suggested that the MR cell is the site of action of aldosterone¹¹. Although the G cells may well participate in the hormone-induced sodium and hydro-osmotic fluxes¹², our data indicate a unique role for the MR cells in the hormonal regulation of transport in this tissue. Indeed, the unique stellate arrangement of G cells around the less numerous MR cells¹³ raises the possibility that the two cell types may act cooperatively in the tissue's response to peptide and steroid hormones.

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Cytochalasin B increases collagenase production by cells *in vitro*

COLLAGENASE is one of the major enzymes involved in the remodelling of connective tissues in normal and pathological states. Specific collagenases have been isolated from the culture media of many tissues *in vitro*¹ and have been found in culture media from various cells in monolayer culture, including rabbit synovial fibroblasts², guinea pig macrophages^{3,4} and human skin fibroblasts⁵. For a better understanding of the mechanisms by which collagenases are synthesised and released from cells, studies have focused on agents that stimulate these processes. Endotoxin⁶ and lymphokines⁴ induce release of collagenase from guinea pig macrophages, and a sustained release of collagenase and neutral protease from rabbit synovial fibroblasts was produced by phagocytosis of polystyrene latex particles⁷. It seems likely that all these agents affect cell membrane function, and so we have tested the effect on collagenase production by rabbit synovial fibroblasts of cytochalasin B (CB), which perturbs membranes in ways similar to phagocytosis⁷.

Fibroblast-like cells grown out from primary explants of rabbit synovium were dissociated mechanically or by a trypsin-EDTA mixture and then grown to confluence in glass prescription bottles in Dulbecco's modified Eagle's medium (DMEM) fortified with 10% foetal calf serum (FCS). Cells from the sixth to fourteenth passage generations were used. Human cells derived from synovial membrane were obtained in similar fashion from tissue provided by Mr Alan Murley, Orthopaedic Section, Addenbrooke's Hospital, Cambridge. Human skin fibroblasts grown from two normal children and from a child with the Hurler syndrome (type I mucopolysaccharide storage disease) were the gift of Dr William Sly, Department of Paediatrics, Washington University, St Louis, Missouri.

For studies with CB, cells were passaged into Nunclon plastic cell culture dishes (diameter 25 mm) and used when they approached confluence. As Figure 1 shows there was a large increase in collagenase activity in culture medium from CB-treated rabbit cells ($1 \mu\text{g ml}^{-1}$). CB caused cells to retract cytoplasm around nuclei, leaving thin cell processes extending in multiple directions, and this effect was greater in cells exposed to $10 \mu\text{g ml}^{-1}$; at $0.1 \mu\text{g ml}^{-1}$, CB caused

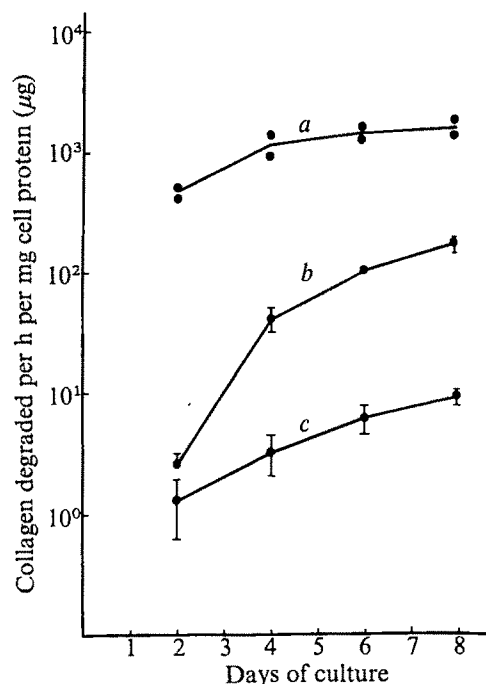


Fig. 1 Stimulation by CB of collagenase in cultures of rabbit synovial fibroblasts. CB was made up in a stock solution in DMSO (5 mg ml^{-1}) and diluted in DMEM before use. DMSO (0.1–1%) had no effect on collagenase production or activity. Medium containing 10% FCS was replaced with serum-free medium at time 0 and subsequently collected every 48 h. Collagenase activity was assayed using ^{14}C -labelled collagen fibrils as substrate (reaction mixture: $100 \mu\text{l}$ 0.2% collagen, $80 \mu\text{l}$ culture medium, $20 \mu\text{l}$ 0.01 M *p*-chloromercuribenzoate and $100 \mu\text{l}$ 0.1 M Tris-HCl, pH 7.6, 0.03 M CaCl_2). Incubation was for 18 h at 37°C . The protein content, determined by the method of Lowry *et al.*¹⁴, of cells adhering to dishes at the end of the experiment did not differ significantly among groups. Duplicate assays were performed on all samples. Values for CB at $10 \mu\text{g ml}^{-1}$ represent two dishes (a). Eight dishes received CB at $1.0 \mu\text{g ml}^{-1}$ (b) and there were four control dishes (c); means \pm s.e.m. are shown for control samples, and those treated with CB at $1.0 \mu\text{g ml}^{-1}$. The cumulative enzyme release from the cells treated with CB at $1.0 \mu\text{g ml}^{-1}$ was different from the controls ($P < 0.01$).

no morphological changes and no increase in collagenase activity. Concentrations of CB $> 10 \mu\text{g ml}^{-1}$ in the absence of FCS were toxic to cells; many became detached from the dishes. It is not essential to preincubate cells with CB in the presence of FCS to stimulate collagenase release, but removal of CB from medium resulted in a prompt fall in collagenase release to control levels.

Studies with the human fibroblasts have given essentially similar results (Table 1). Dissociated cells were added to duplicate dishes in dilutions of zero (2×10^5 cells ml^{-1}), 1:1 (10^5 cells ml^{-1}) and 1:3 (0.5×10^5 cells ml^{-1}). CB ($1.0 \mu\text{g ml}^{-1}$) was then added only to the cells diluted 1:1. More collagenase was released from untreated Hurler cells than from untreated normal cells, as noted by others (unpublished results of Z. W., W. Sly and J. T. Dingle). The fractional increase over control levels of enzyme release induced by CB was, however, greater in the fibroblasts derived from normal skin.

The cumulative collagenase activity released from human fibroblast-like cells (eighth passage) derived from synovium removed during insertion of a prosthetic hip in a 75-yr-old woman with degenerative arthritis was determined, using a protocol similar to that described in the legend of Fig. 1. In the 6 d of culture after addition of serum-free medium containing CB ($1.0 \mu\text{g ml}^{-1}$) there was a cumulative mean collagenase activity (U) in four dishes of $1,423 \pm 78$ (s.e.m.) compared with the lower level of 557 ± 162 U released from untreated cells ($P < 0.01$).

Possible explanations for the increased collagenase

Table 1 Collagenase activity in cell culture medium from human fibroblasts

Cells	Cell dilution* and CB addition	Cumulative collagenase during 4 d (U†)
Normal (line 236)	Zero (no CB)	658
	1:1 (CB, 1 µg ml ⁻¹)	2,967
	1:3 (no CB)	580
Normal (line 235)	Zero (no CB)	270
	1:1 (CB, 1 µg ml ⁻¹)	3,234
	1:3 (no CB)	413
Hurler	Zero (no CB)	1,455
	1:1 (CB, 1 µg ml ⁻¹)	5,357
	1:3 (no CB)	1,805

The number of cells initially seeded into the dishes did not change the collagenase activity markedly, but the additional of CB (1 µg ml⁻¹) produced a large increase with all three types of cells. See text and the legend to Fig. 1 for experimental details and assay conditions.

* Disaggregated cells (2 × 10⁵ cells per ml in DMEM with 10% FCS) were plated in duplicate plastic dishes in the dilution shown. When the dishes with the lowest dilution had reached confluence, serum-free medium was added to each dish and CB was added only to those dishes plated originally at 1:1 dilution.

† 1 Unit = 1 µg collagen degraded per h per mg cell protein.

activity in the medium brought about by CB are numerous, and include increased release of preformed collagenase already stored intracellularly, activation of a zymogen of the enzyme, destruction or blockage of an inhibitor of collagenase secreted by the cells, and (or) an increased *de novo* synthesis of collagenase. The following experiment, one of several essentially similar experiments, was carried out to show whether untreated cells had more enzyme stored intracellularly than treated cells, and to test the dependence of collagenase release on new protein synthesis. Six dishes were inoculated with rabbit synovial cells (twelfth passage); three of these were exposed to CB (1.0 µg ml⁻¹). Cycloheximide (Sigma) was added to one control and to one CB-treated dish after collection of medium on day 2 of culture. After medium was collected on day 4, the cells of each dish were washed, dispersed and a portion was counted. The remaining cell suspension was frozen and thawed rapidly (×4) and assayed for collagenase, as was the culture medium from each dish. Table 2 shows clearly that cycloheximide diminished the collagenase production in the treated cells (by more than 90%); enzyme activity in the medium from control cultures was very weak and too near baseline values to make comparisons among dishes. Little difference was found in collagenase activity between control and CB-treated cell lysates.

There is evidence that latent collagenase in culture medium from fibroblasts can be activated by incubation

Table 2 Effect of cycloheximide on stimulation of collagenase by CB

		Collagenase in medium (U*)		Collagenase in cell lysate (U*)
		Days 0-2	Days 2-4	
Control cells	1	107	234	387
	2	49	87	181
	3	140	98†	157‡
CB-treated cells	1	2,018	2,196	372
	2	2,150	1,916	270
	3	2,506	226†	160‡

Rabbit cells were grown for 4 d with or without CB, and one dish from each group was also treated with cycloheximide (10 µg ml⁻¹) during the second 2-d culture period. Cell lysates were prepared at the end of the 4-d period.

* 1 Unit = 1 µg collagen degraded per 10 h per 10⁶ cells.

† Cycloheximide during days 2-4 of culture. Cycloheximide (1-100 µg ml⁻¹) had no effect on collagenase activity when added directly to assays.

‡ Cell lysates from cells to which cycloheximide had been added during culture days 2-4.

with small amounts of trypsin⁵. Whether this signifies the presence of a zymogen, such as that reported for the collagenase in tadpole tails⁸, mouse bone⁹ and polymorphonuclear leukocytes^{10,11}, or destruction or saturation of inhibitory substances^{12,13} is not known. To test the possibility that CB activated a zymogen or inactivated inhibitors in these cultures, media pools from six CB and six control cultures of rabbit synovial fibroblasts (fourteenth passage) were incubated with crystalline trypsin (0.5 µg trypsin per 8.5 µg medium protein) for 1 h at 37 °C. An excess of soy bean trypsin inhibitor was then added and aliquots of each sample were assayed in quadruplicate. The following results (expressed as c.p.m. ¹⁴C-collagen solubilised per 10 h per ml) were obtained: control (no trypsin), 3.4; control (trypsin), 4.1; CB (no trypsin), 870; CB (trypsin), 1,207. Viscometric analysis (27 °C) of the trypsin-activated CB medium with subsequent electrophoresis on polyacrylamide gels revealed typical cleavage of soluble collagen into A (75%) and B (25%) fragments. Similar data have been obtained in other experiments, and we suggest, therefore, that at least a part of the collagenase activity stimulated by CB may be latent in a zymogen or inhibited form.

The precise mechanism by which CB stimulates the production of both active and latent collagenase is unknown, but our results support the idea that the important determinants which, when triggered, activate collagenase production and release by cells are located in cell membranes. Studies underway in our laboratory showing that aminophyllin stimulates collagenase release from fibroblasts (unpublished results of E. Cartwright, E. D. H. and J. J. R.) may implicate cyclic nucleotides in the control of the synthesis and release of non-lysosomal enzymes. Whatever the precise mechanism of action of CB, its use with human and animal cell cultures will be valuable for investigating the role of collagenases in connective tissue diseases.

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Induction of synthesis of bacterial protein by excretory product of the alga *Chlamydomonas reinhardtii* γ-1

MANY examples are known of interspecies relationships between widely different organisms in natural communities¹. The benefits that one or more members of a population obtain as a result of their association have been described

mainly in terms of the nutritional value of the relationship, measured in terms of growth rate, metabolic activities, or the ability to survive in a selective environment¹⁻³. We have discovered an apparent commensal relationship between the alga *Chlamydomonas reinhardtii* y-1 and a bacterium, tentatively identified as a coryneform bacterium. As a result of their association, the synthesis of a single protein is stimulated to a remarkable extent within the bacterial cells, whereas the algal cells apparently obtain neither benefit nor detriment from the relationship.

This discovery was facilitated by the observation that ³H-leucine (40–60 Ci mmol⁻¹), when added to mixed cultures of these two organisms to a concentration near 10⁻⁷ M, is selectively incorporated only into proteins synthesised within the bacterial cells. Figures 1 and 2 show the electrophoretic patterns of radioactivity obtained after ³H-leucine was added to pure, dark-grown cultures of the bacteria (Fig. 1a) and *C. reinhardtii* y-1 (Fig. 1b), and to a mixed, dark-grown population of both organisms (Fig. 2) after 1 h of exposure to light. Very little radioactivity was

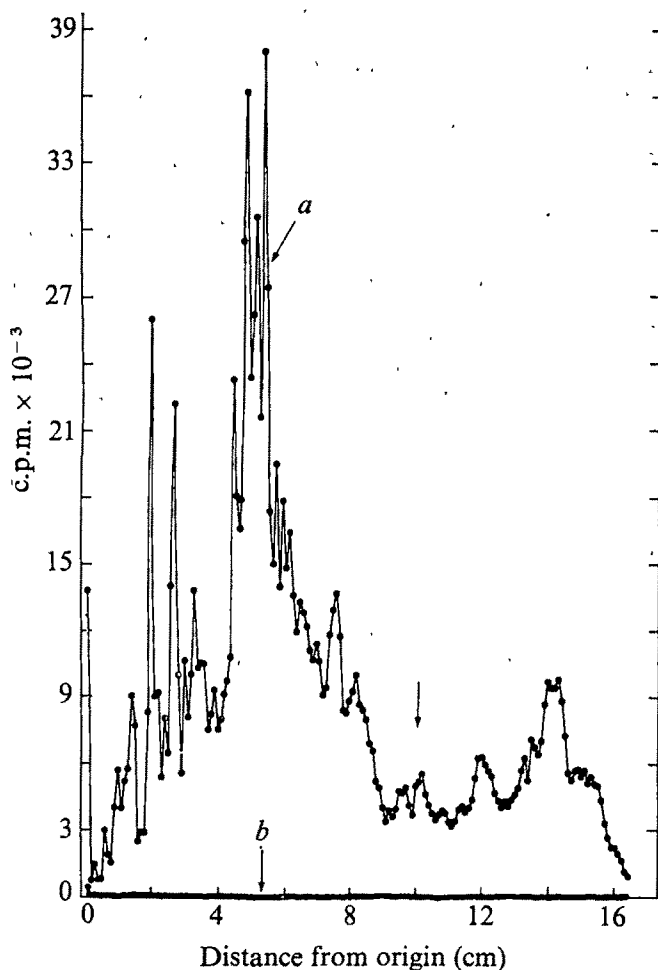


Fig. 1 Electrophoretic analysis of the incorporation of ³H-leucine into total polypeptides of bacteria (a) and *C. reinhardtii* y-1 (b), each grown separately in pure culture. Bacterial cells were grown in the dark in the algal medium⁶ containing 0.5% yeast extract and 0.5% peptone. The cells were washed twice and resuspended in the algal medium to a density of 0.1A₄₅₀. Dark-grown cells of both organisms were exposed to light for 1 h, and ³H-leucine (4 μCi ml⁻¹) was added to each culture. After 1 h incubation cells from each culture were collected by centrifugation. Bacterial cells were incubated for 45 min in 25 mM Tris-HCl (pH 7.6) containing 1 mM EDTA, lysozyme 0.25 mg ml⁻¹, and chloramphenicol 25 μg ml⁻¹. In these conditions the bacterial cells swell but do not lyse. The cells were collected by centrifugation and frozen. Procedures for electrophoresis of total cellular proteins and for determination of the patterns of radioactivity were as described¹²⁻¹⁴.

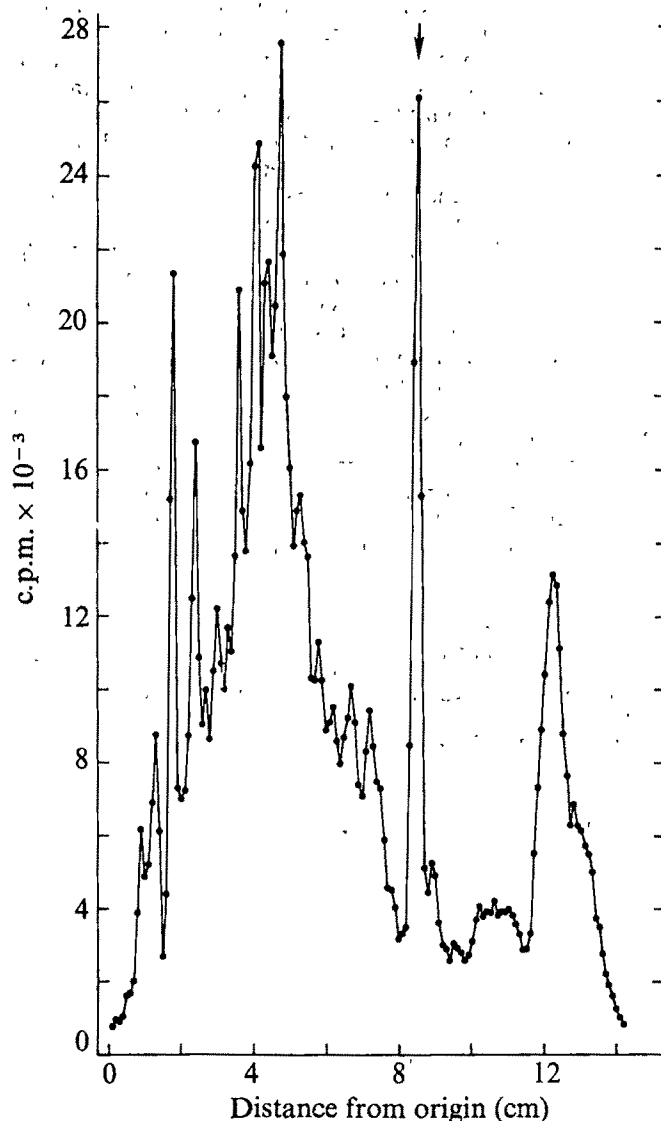


Fig. 2 Electrophoretic analysis of the incorporation of ³H-leucine into total polypeptides of a mixed population of bacterial and algal cells labelled in the light. Bacteria were grown as described in Fig. 1 and mixed with etiolated cells of *C. reinhardtii* y-1. The mixed population was exposed to light for 1 h, and incubated for an additional 1 h with ³H-leucine (4 μCi ml⁻¹) in the light. Cells of both organisms were incubated with lysozyme and total proteins were prepared for electrophoresis as described in Fig. 1.

detected in gels containing total proteins of the algal cells grown in pure culture (Fig. 1b). With the exception of a single polypeptide fraction (arrows), however, the pattern of total radioactivity for the bacterial cells alone (Fig. 1a) resembled that for mixed cells (Fig. 2). This incorporation of leucine by the bacterial cells was resistant to the action of cycloheximide, but was nearly abolished by inhibitors of 70S ribosomes⁴. Furthermore, the incorporation was blocked by inhibitors of prokaryotic RNA synthesis⁴.

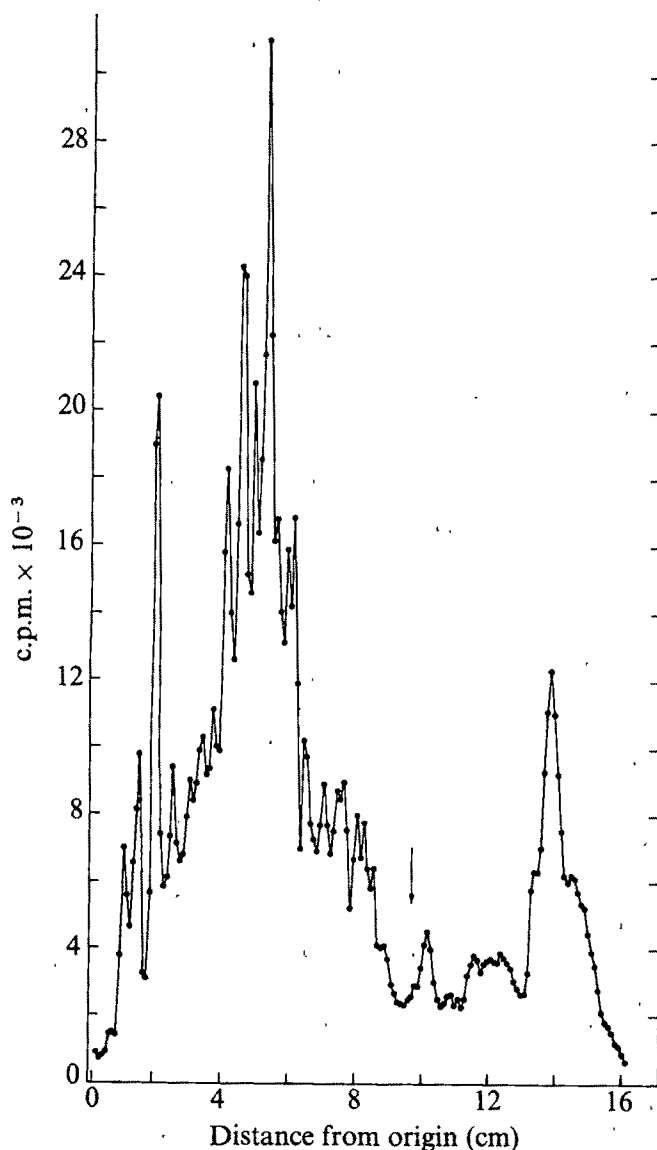
Leucine seems to be poorly transported across the plasma membrane of *C. reinhardtii*, as a several hundredfold increase in the extracellular concentration of this amino acid was required to obtain significant labelling of the algal proteins⁴. The resulting pattern of radioactivity for algal cells labelled with ³H-leucine (1 Ci mmol⁻¹, 3 × 10⁻³ M in the medium) bore no resemblance to the bacterial pattern shown in Fig. 1a.

The prominent peak of radioactivity in the pattern for mixed cultures (Fig. 2, arrow) corresponds to a polypeptide

with a molecular weight of about 21,500. Synthesis of this polypeptide within the bacterial cells was observed when dark-grown cultures of a mixed population of bacterial and algal cells was exposed to light (Fig. 2), but was not observed when a mixed culture was labelled in the dark (Fig. 3) without exposure to light. Nor was this polypeptide synthesised when dark-grown bacterial cells were exposed to light in the absence of the algal cells (Fig. 1a), suggesting that the photoreceptor for synthesis of this polypeptide resides within the algal cells. Thus, the absorption of light by etiolated cells of *C. reinhardtii* y-1 elicits a response that stimulates the synthesis of a single protein within this bacterium as the result of their association.

Algae are known to excrete a wide variety of substances⁵⁻⁷, and commensal relationships have been reported in which the growth of bacteria depended on the excretion of oxidisable substrates by algal cells³. To test for excretion of an inducing substance by *C. reinhardtii* y-1, a pure culture of dark-grown algal cells was exposed to light, and after 90 min of illumination, the algal cells were removed by centrifugation. Synthesis of the inducible protein was

Fig. 3 Electrophoretic analysis of the incorporation of ³H-leucine into total polypeptides of a mixed population of bacterial and algal cells labelled in the dark. Dark-grown cells of both organisms were mixed and incubated with ³H-leucine (4 μ Ci ml⁻¹) for 1 h without exposure to light. After treatment with lysozyme, total proteins of the cell mixture were prepared for electrophoresis as described in Fig. 1.



stimulated when the bacteria were suspended in this medium. No induction of the synthesis of this protein, however, was detected when the bacteria were suspended in medium obtained in the same way from a mixed population of the two organisms. These results indicated that in response to light the etiolated algal cells released a limited amount of the inducing factor into the medium, and that this factor was quantitatively used by the bacterial cells.

Although we have not identified this factor, its production may be related to the breakdown of starch in etiolated *C. reinhardtii* y-1. In this alga, starch breakdown requires continuous illumination and is maximal during the lag phase of chlorophyll synthesis⁸. The time course of synthesis of the inducible protein within the bacteria during continuous illumination of mixed cultures also reached a maximum during the lag phase of chlorophyll synthesis. Thereafter, the rate declined until synthesis of this protein was barely detectable when the algal cells had become fully green (data to be published elsewhere).

The coryneform bacteria contain a large number of plant saprophytes found in soil and water environments⁹⁻¹¹. Many species within this group require vitamins and other essential organic molecules that are normally obtained from their natural habitats⁹⁻¹¹. The coryneform bacteria used in these studies were isolated as contaminants of our y-1 strain of *C. reinhardtii*. They grow poorly in the defined algal medium containing acetate as a carbon source⁸, and on agar they form transparent colonies not visible to the unaided eye.

The bacteria could not be separated from the algal cells by sucrose gradient or differential centrifugation techniques⁴, because of aggregation of the bacteria in liquid media. Lysozyme treatment was required to lyse the bacteria sufficiently to release proteins for gel electrophoresis. We previously concluded that leucine had selectively entered the mitochondrial pool of amino acids within the algal cells, as the latter do lyse in these conditions without lysozyme treatment⁴.

As we do not know of any benefit derived from synthesis of the inducible protein within the bacteria, at present their relationship with *C. reinhardtii* y-1 must be considered commensal only in so far as one member seems to be affected by the association. True commensalism has been found between bacteria and other algal and yeast cells based on the nutritional requirements of the bacteria^{2,3}. This is the first report, however, that such an interaction may control specific gene expression within one member of the association. Further studies are in progress to determine the identities of the algal induction factor and the protein synthesised by these bacteria in response to this factor.

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Kinetics of inactivation of histone mRNA in the cytoplasm after inhibition of DNA replication in synchronised HeLa cells

In many eukaryotic cells DNA replication and histone synthesis proceed synchronously¹⁻⁶. Interruption of DNA synthesis in synchronised HeLa cells results in an immediate decrease of histone synthesis as measured by incorporation of labelled amino acids into histones^{2,3} or by following the completion of nascent histone polypeptide chains on polyribosomes *in vitro*⁵.

Recent long-term labelling experiments by Perry and Kelley⁷ suggest that in mouse L cells the lifetime of histone mRNA is of the order of the length of one S-phase (that is about 11 h). If, however, DNA replication is blocked with cytosine arabinoside or hydroxyurea, labelled histone mRNA selectively disappears from polyribosomes between 30 and 60 min after addition of these drugs to the cells⁵⁻⁷. In contrast, actinomycin D (5 $\mu\text{g ml}^{-1}$) only slightly decreases histone synthesis during the first hour of its action⁸.

Recently we have introduced a method of titrating the amount of biologically active histone mRNA by its transla-

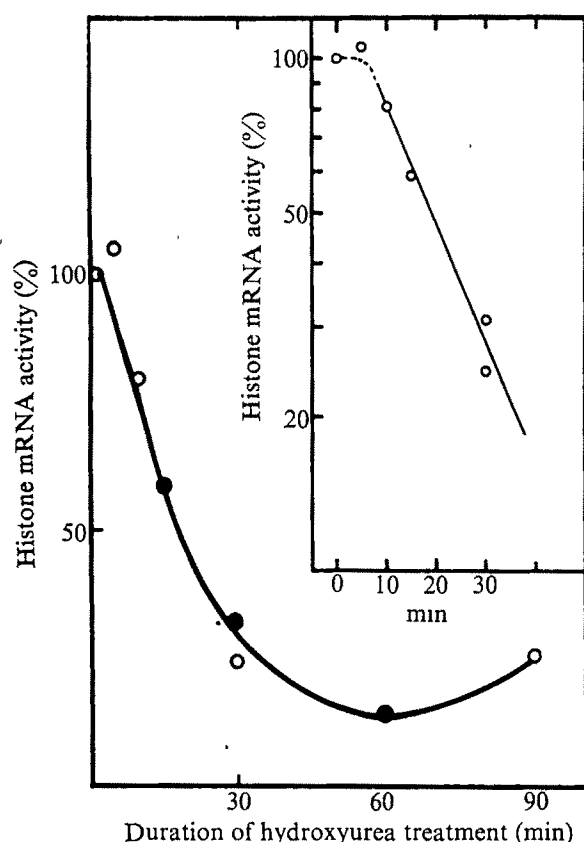


Fig. 1 Inactivation of polyribosomal histone mRNA after termination of DNA synthesis. Thymidine-synchronised cells 3 h in S-phase were treated with 3 mM hydroxyurea for different times and lysed in hypotonic buffer. Polyribosomes were isolated as described¹⁰, resuspended in 35 mM Tris-HCl (pH 7.5), 10 mM EDTA, 0.1 M NaCl, 0.5% sodium dodecyl sulphate and the RNA was extracted with buffer-saturated phenol and phenol-chloroform (1:1, v/v)¹⁰. The RNA was passed over poly(U)-Sepharose equilibrated with 10 mM Tris-HCl (pH 7.5) containing 0.5 M KCl: RNA not adsorbed to poly(U)-Sepharose was fractionated on sucrose gradients and histone mRNA translated in a rabbit reticulocyte lysate as described¹⁰. The amount of mRNA was calculated from the radioactivity incorporated into protein according to the equation $(A-B)/a$; A and B being (c.p.m. in CM-2 $\times 100$)/(c.p.m. in CM-1) from lysates incubated in the presence or absence of histone mRNA, respectively; a being (μg of polyribosomal poly(A)(-)-RNA from which histone mRNA was recovered). Open and closed circles represent data from two separate experiments. The insert is a semi-logarithmic plot of the data.

tional capacity in a rabbit reticulocyte lysate⁹. Using this method we showed that in S-phase of synchronised HeLa cells about 80% of functional histone mRNA was lost from polyribosomes within 60 min of inhibition of DNA synthesis whereas actinomycin D for 1 h only slightly decreased histone mRNA activity⁹. We describe experiments here showing that these RNA species decay from the cytoplasm with an approximate half life of 13 min after interruption of DNA replication with hydroxyurea.

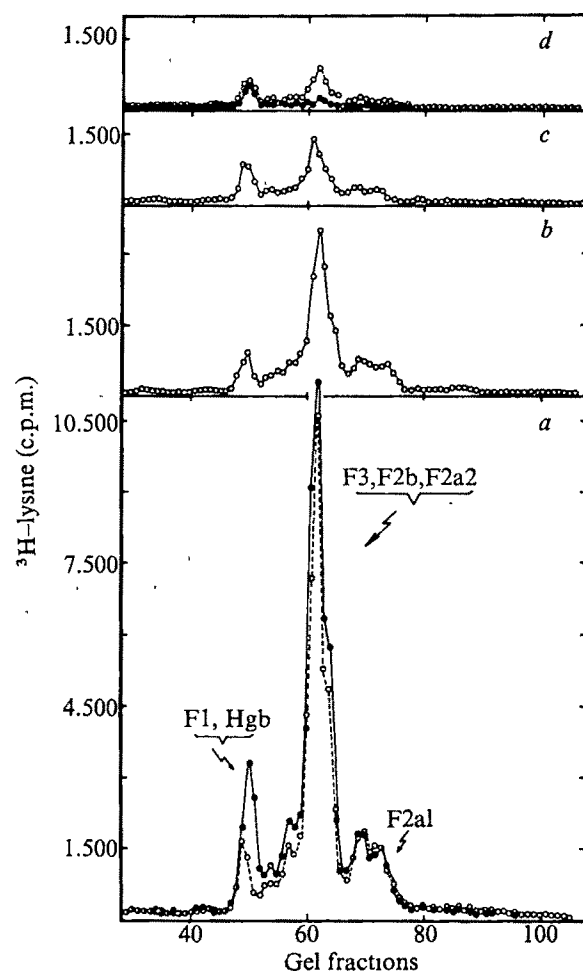


Fig. 2 Polyacrylamide gel electrophoresis of acid-soluble proteins (CM-2 fraction) synthesised in a reticulocyte lysate under the direction of histone mRNA from cells 3 (○) and 4 (●) h in S-phase (a) and from 3-h S-phase cells treated with 3 mM hydroxyurea for 15 min (b), 30 min (c) and 60 min (d, ○). CM-2 proteins from a lysate incubated without added mRNA are represented in d (●). Proteins were dissolved in 6 M urea containing 0.5 M 2-mercaptoethanol, incubated for 30 min at room temperature and electrophoresed in 15% acrylamide gels containing 2.5 M urea¹⁴. Gels were sliced and radioactivity measured as described¹⁰.

HeLa S-3 cells were grown in suspension culture in Eagle's minimum essential spinner medium supplemented with 5% calf serum. Cells were synchronised by a double block with 2 mM thymidine and allowed to enter S-phase by resuspension in fresh medium at a concentration of 3×10^5 – 4×10^5 cell ml^{-1} (ref. 10). Three and four hours after entering into S-phase the cells synthesise histones at maximal rate⁶. Cells were then treated for different times with 3 mM hydroxyurea which results, within less than 5 min, in an inhibition of thymidine incorporation by more than 98%. Polyribosomal RNA was isolated and passed over poly(U)-Sepharose columns to purify it from poly(A)-containing mRNA. Histone mRNA lacking poly(A) at the 3'-OH end¹¹ was then separated on sucrose gradients and quantitated by its translation in a reticulocyte lysate as described previously^{8,9}. Acid-soluble proteins of the lysate were separated by CM-cellulose chromatography into

two fractions, CM-1 which predominantly contains globin and CM-2 containing the histones¹⁰.

From Fig. 1 it can be deduced that as early as 10 min after a blockade of DNA replication about 20% of functional histone mRNA is lost from polyribosomes in comparison to the mRNA content of 3-h S-phase cells. Thirty and 60 min after arrest of DNA synthesis less than 30% and 15% respectively of active histone mRNA are left on polyribosomes. Further treatment with the blocking agent did not change this value significantly. A further increase of polyribosomal histone mRNA by about 20–30% was observed between the third and fourth hour after entry into S-phase which subsequently decreased again (data not shown). It should be pointed out that active histone mRNA does not survive unbound to ribosomes in the cytoplasm after DNA synthesis is stopped^{9,12}.

The gel electrophoretic profiles show that synthesis of all histone fractions is equally inhibited following the block of DNA replication (Fig. 2). It can also be seen in Fig. 2 that CM-2 proteins from reticulocyte lysates incubated without added mRNA contain labelled material which predominantly coelectrophoreses with globin (Fig. 2d, ●, fractions 48–52). Therefore, such control lysates had to be taken into account in the calculation of histone mRNA activity (see legend to Fig. 1).

If it is assumed that after interruption of DNA replication the flow of new histone mRNA from the nucleus to the cytoplasm immediately ceases (as a result of an inhibition of either the transcription of histone genes, the processing of histone mRNA in the nucleus or its transport into the cytoplasm) the disappearance of histone mRNA from the cytoplasm is a function mainly of the degradation (or inactivation) of that RNA. From a semilogarithmic plot of the data presented in Fig. 1 it can be deduced that, after a short lag of about 5 min, histone mRNA is inactivated according to first-order kinetics with a

half life of about 13 min (insert in Fig. 1). The short lag possibly results from the time required for hydroxyurea to penetrate the cells and to interrupt subsequent flow of histone mRNA from the nucleus to the cytoplasm.

Butler and Mueller¹³ recently reported that cycloheximide prevents the inhibition of histone mRNA synthesis by hydroxyurea. We measured the polyribosomal mRNA in cells which were treated with cycloheximide and hydroxyurea simultaneously and found that although DNA replication was completely blocked by the two drugs histone mRNA survived on polyribosomes in these experimental conditions (Fig. 3). Since cycloheximide 'freezes' polyribosomal structures this study indicates that histone mRNA in cells treated in this manner is protected from degradation by its binding to ribosomes. This implies that a step in the initiation of histone mRNA translation may be inhibited in the absence of DNA replication so that the mRNA becomes susceptible to cellular nuclease(s). Alternatively, the inactivation of histone mRNA in the cytoplasm after cessation of DNA synthesis could be linked in some unknown manner to a process depending on protein synthesis.

The extremely short half life of histone mRNA in the absence of DNA replication described here is consistent with the data presented by Perry and Kelley⁷ which indicate that in mouse L cells the decay of histone mRNA increases nearly linearly with the age of these RNA species. From all the known experimental results it is now well substantiated that histone mRNA is present on polyribosomes only during the DNA replication period of the cell cycle^{5-7,10,12,15}. These RNA species are synthesised during the first few hours of S-phase starting with the initiation of DNA replication^{6,10}, they are stable during S-phase⁷ and are destroyed with a half life of about 13 min after termination of DNA synthesis. The shut-down of histone synthesis at the end of S-phase is therefore the consequence of an inactivation of cytoplasmic histone mRNA which seems to involve a specific translational control mechanism operating at the initiation of histone mRNA translation.

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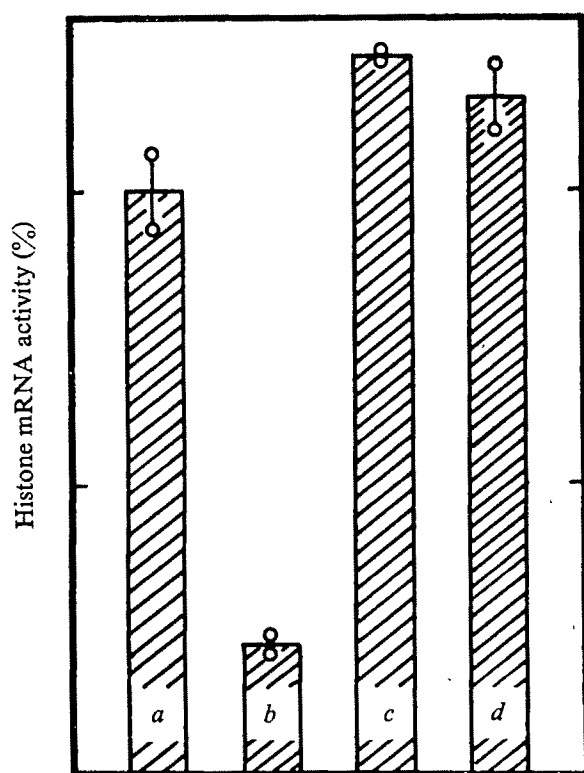


Fig. 3 Effect of cycloheximide on hydroxyurea-induced inactivation of histone mRNA. 600-ml spinner cultures of synchronised cells 3 h in S-phase were untreated (a) or treated for 45 min with either 3 mM hydroxyurea (b), 10 μM cycloheximide (c) or with both drugs simultaneously (d). In the latter case cycloheximide was added to the cultures 3 min before hydroxyurea. Two minutes before collecting the cells, cycloheximide was also added to cultures a and b. The data represent two separate experiments.

In vitro transcription of a late class of phage SP01 genes

Bacillus subtilis phage SP01 directs the synthesis of at least six temporally defined classes of phage-specified transcripts¹. Synthesis of the two earliest classes of phage RNA (e and em) does not require phage protein synthesis, while the synthesis of middle (m and m₁) and late (m₂ and l) transcripts requires the protein product of regulatory gene 28 and the products of regulatory genes 33 and 34, respectively^{2,3}. The discovery of SP01-induced polypeptides associated with *B. subtilis* RNA polymerase suggested that the products of SP01 regulatory

genes could control transcription by interacting directly with the host RNA polymerase^{4,5}. In support of this idea, we reported⁶ that a form of RNA polymerase from SP01-infected cells copies middle RNA almost exclusively from the heavy (H) strand of native SP01 DNA, the DNA strand from which middle and late genes are transcribed *in vivo*. This enzyme contains an SP01-induced polypeptide termed IV (molecular weight 26,000) that is now known to be the product of regulatory gene 28 (T. Fox, R. L. and J. P., manuscript in preparation). Accurate middle gene transcription by this phage-modified RNA

polymerase does not require σ factor but is apparently dependent on a newly described host subunit of RNA polymerase called δ (molecular weight 21,500)⁶. Other workers have also reported the preferential synthesis of middle RNA by RNA polymerase from phage-infected *B. subtilis*^{7,8}. Here we report that in the presence of host δ protein a form of RNA polymerase containing phage-induced polypeptides termed V (molecular weight 24,000) and VI (molecular weight 13,500) asymmetrically transcribes a class of SP01 late genes *in vitro*.

Two forms of RNA polymerase designated enzymes B and C were purified from SP01-infected bacteria as previously described⁶. In addition to the subunits of core RNA polymerase (β' , β , α and ω), enzyme C contained phage-induced subunits V and VI, while enzyme B contained phage-induced subunit IV and traces of VI (Fig. 1). Both enzymes lacked the host δ protein and copied RNA with only a small preference for the H strand of native SP01 DNA (Fig. 2). In the presence of δ protein purified from uninfected bacteria (Fig. 1), however, enzymes B and C copied RNA predominantly from strand H (the H/L hybridisation ratios increased from 1.7 to 9.4 and from 1.2 to 8.6, respectively; Fig. 2). As previously reported⁶, δ had little effect on the strand selectivity of transcription by core enzyme from uninfected cells (the H/L ratio increased from 1.2 to 2.0; data not shown). Thus in the presence of δ ,

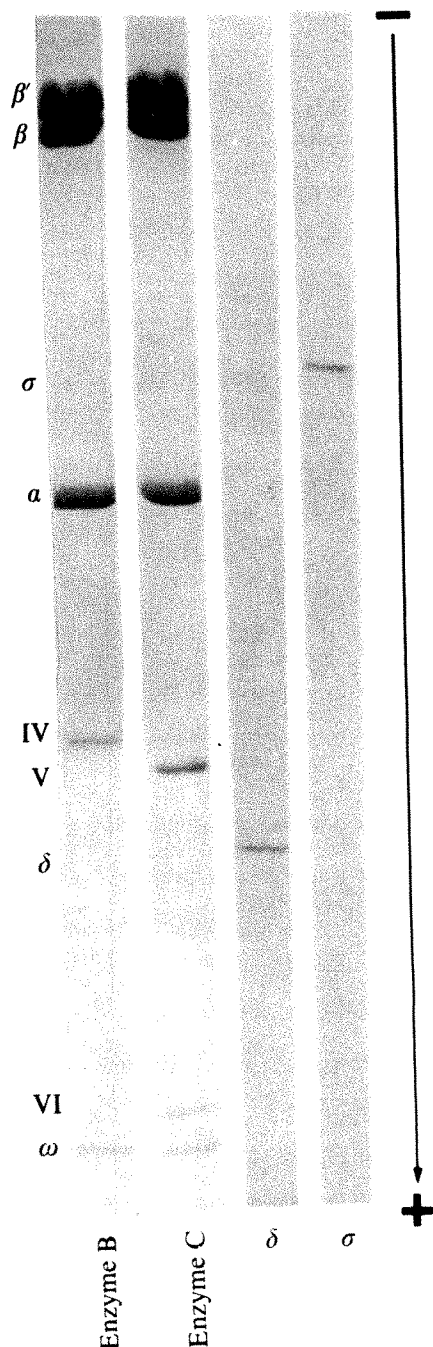


Fig. 1 SDS polyacrylamide slab gel electrophoresis of enzyme B, enzyme C, δ and σ . Enzymes B and C were purified as described previously⁶. δ and σ were dissociated from purified holoenzyme⁹ from uninfected *B. subtilis* by chromatography on phosphocellulose⁹, dialysed against buffer C (ref. 4) containing 0.01 M EDTA and 0.02 M KCl, and then applied to a poly(C)-cellulose column¹⁰. δ eluted in the flow-through while σ , which adhered to the column, was eluted with buffer containing 8 M urea. Electrophoresis was on a 25-cm long slab gel containing sodium dodecyl sulphate in a Tris-glycine buffer and a 7–15% gradient of polyacrylamide¹¹.

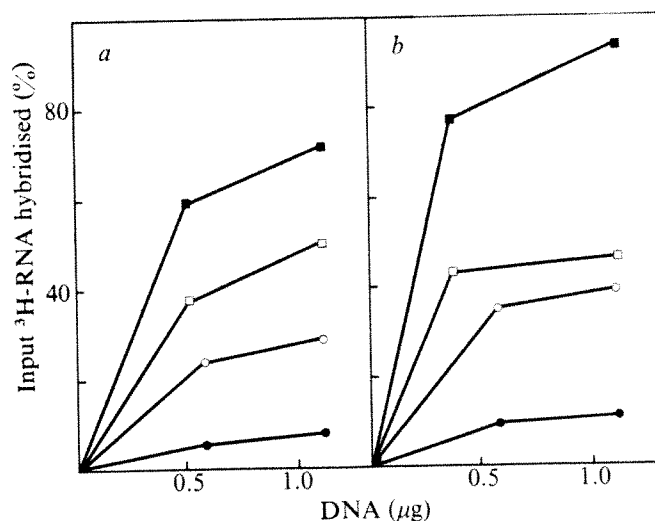


Fig. 2 Effect of δ on the strand selectivity of transcription by enzymes B and C. Radioactive RNA was synthesised *in vitro* by 3.5 μ g of enzyme B and 2.0 μ g of enzyme C in the absence (open symbols) and presence (closed symbols) of 0.4 μ g of purified δ (Fig. 1). Hybridisation reactions contained the indicated amounts of H strand (\square , \blacksquare) and L strand (\circ , \bullet) DNA and the following 3 H-RNAs in 125 μ l of $2 \times$ SSC: a, RNA synthesised by enzyme B (\square , \circ) (input, 25,200 c.p.m.; background, 490 c.p.m.); RNA synthesised by enzyme B + δ (\blacksquare , \bullet) (input, 22,700 c.p.m.; background, 1,100 c.p.m.). b, RNA synthesised by enzyme C (\square , \circ) (input, 14,500 c.p.m.; background, 540 c.p.m.); RNA synthesised by enzyme C + δ (\blacksquare , \bullet) (input, 11,700 c.p.m.; background, 280 c.p.m.). The experimental procedures for the synthesis of RNA, preparation of DNA strands and the hybridisation of RNA were described previously⁶.

enzymes B and C directed highly asymmetric transcription of SP01 DNA.

Since enzymes B and C contained different phage-induced polypeptides, it seemed possible that in the presence of host δ protein each phage-modified RNA polymerase was transcribing asymmetrically a different class of SP01 genes. To compare the classes of RNA synthesised by enzyme B + δ and enzyme C + δ , radioactive RNA synthesised *in vitro* was hybridised to denatured SP01 DNA in the presence of unlabelled RNA from cells collected at early (CAM), middle (10') and late (30') times in the lytic cycle. (The early RNA was purified from cells that had been treated with chloramphenicol to block phage protein synthesis.) The experiment of Fig. 3c shows that the hybridisation of RNA synthesised by enzyme B + δ was more effectively

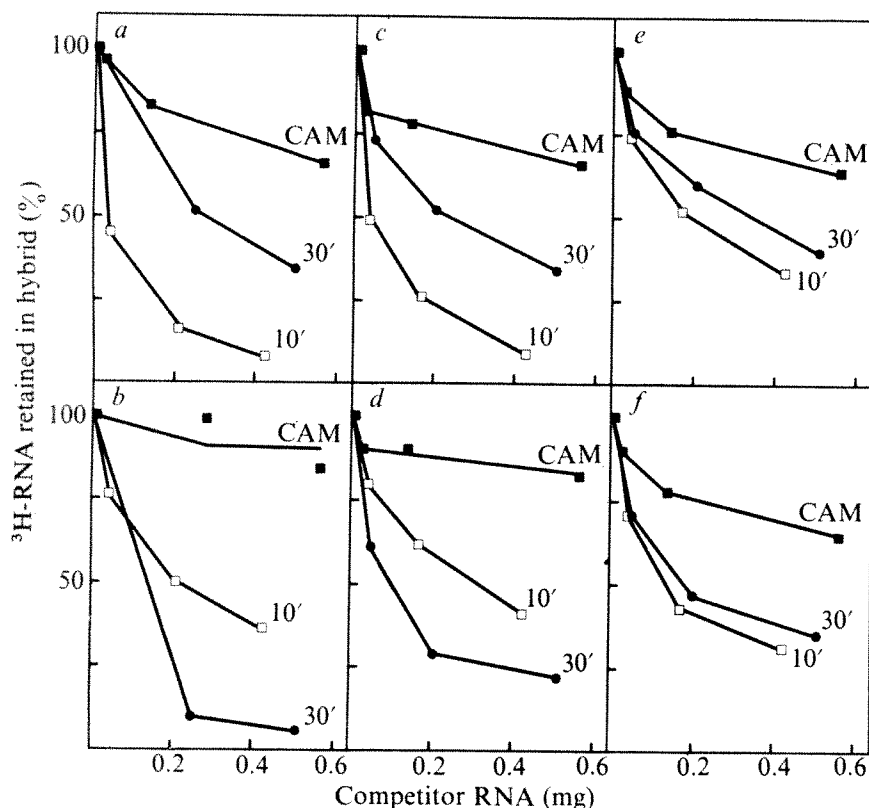


Fig. 3 Hybridisation competition of ^3H -RNAs by competitor RNAs isolated at various times after infection. Hybridisation reactions contained 2.5 μg of denatured SP01 DNA, the indicated amounts of unlabelled competitor RNAs and the following ^3H -RNAs in a total volume of 200 μl of $2\times\text{SSC}$. *a*, Middle SP01 RNA pulse labelled *in vivo* 8–10 min after infection; 33% of the input RNA (21,000 c.p.m.) hybridised in the absence of competitor. *b*, Late SP01 RNA pulse labelled *in vivo* 27–29 min after infection; 18% of the input RNA (23,000 c.p.m.) hybridised in the absence of competitor. *c*, RNA synthesised *in vitro* by enzyme B+ δ ; 39% of the input RNA (9,800 c.p.m.) hybridised in the absence of competitor. *d*, RNA synthesised *in vitro* by enzyme C+ δ ; 61% of the input RNA (11,200 c.p.m.) hybridised in the absence of competitor. *e*, RNA synthesised *in vitro* by core polymerase from uninfected *B. subtilis*; 50% of the input RNA (8,100 c.p.m.) hybridised in the absence of competitor. *f*, RNA synthesised *in vitro* by enzyme C; 49% of the input RNA (11,600 c.p.m.) hybridised in the absence of competitor. For each reaction a background of less than 3% of the input was subtracted from the radioactivity that was retained in hybrid. CAM (■) RNA was from bacteria treated with 150 μg of chloramphenicol before infection; 10' (□) RNA and 30' (●) RNA were from cells collected 10 and 30 min after infection, respectively. These competitors were prepared as described previously¹². The experimental procedure for the labelling of RNA *in vivo*, for the synthesis of labelled RNA *in vitro* and for hybridisation of radioactive RNA was as described previously⁶.

inhibited by middle RNA than by the late or early competitors. In fact, the RNA competitors similarly affected the hybridisation of *in vivo* synthesised RNA radioactively labelled at a middle time in the lytic cycle (Fig. 3a). In contrast, the hybridisation of RNA synthesised *in vitro* by enzyme C+ δ was more effectively competed by late RNA than by the middle or early competitors (Fig. 3d); and this competition pattern was similar to that obtained for RNA labelled *in vivo* at a late time in the lytic cycle (Fig. 3b). As further evidence that enzyme C+ δ was preferentially synthesising a class of late RNA, we found that RNA extracted at a late time after infection by a gene 34 mutant was a poor competitor of the *in vitro* product of enzyme C+ δ (data not shown). (This mutant phage fails to synthesise late transcripts m_2 and l (refs 2 and 3).) The specific transcription of SP01 late genes *in vitro* requires the host δ protein since enzyme C alone copied RNA symmetrically (Table 1, ref. 6) from both strands of native phage template (Fig. 2b) and since the hybridisation of RNA synthesised by enzyme C alone was not preferentially competed by late RNA (Fig. 3f). It should be noted, however, that our experiments do not distinguish whether enzyme C+ δ copies all late transcripts (m_2 and l) or only a limited set of late sequences.

Which, if any, of the phage-induced polypeptides present in enzymes B and C direct the transcription of middle or late genes *in vitro*? Enzyme B contains phage-induced subunit IV and traces of subunit VI. It is likely that subunit IV causes the synthesis of middle transcripts (m and/or m_1) *in vitro* since polypeptide IV is now known to be coded by gene 28 (T. Fox, R. L. and J. P., manuscript in preparation), the regulatory gene whose product is necessary for middle gene transcription *in vivo*^{2,3}. While polypeptide IV is synthesised early in the phage lytic cycle, as would be expected of a protein that directs middle gene transcription, polypeptides V and VI first appear at a middle time in the lytic cycle (ref. 4 and T. Fox, unpublished results), a finding consistent with the idea that either or both of these proteins could direct late transcription by enzyme C. A reconstitution experiment with the isolated subunits IV, V and VI will, however, be required to prove that these phage-induced proteins direct specific gene transcription.

Escherichia coli RNA polymerase purified from phage T4-infected bacteria also contains phage-induced polypeptides¹³. Two of these proteins are coded by T4 genes 33 and 55, regulatory genes whose products direct late T4 transcription *in vivo*^{14,15}. Purified *E. coli* RNA polymerase containing these phage-induced subunits does not, however, specifically direct late transcription *in vitro*¹⁶. This could result from the apparent coupling of late transcription to DNA synthesis *in vivo*^{17,18}. It is also possible, however, that late transcription by the T4-modified polymerase requires an unidentified *E. coli* protein equivalent to *B. subtilis* δ in addition to T4 regulatory proteins.

In conclusion, the finding of specific transcription of SP01 middle and late genes by RNA polymerase containing phage-induced subunits supports a model of positive control elaborated by Travers¹⁰ in which phage regulatory proteins direct specific transcription by interacting with RNA polymerase in place of the host σ factor.

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N⁶, O²-dimethyladenosine a novel methylated ribonucleoside next to the 5' terminal of animal cell and virus mRNAs

MODIFIED 5'-terminal structures of the type m⁷G(5')ppp(5')Nm have been identified in various viral^{1–4} and cellular^{5–8} mRNAs. The 5'-terminal modification of mRNA may be carried out by specific guanylyl- and methyltransferases⁹ and the structure seems to be required for efficient *in vitro* translation¹⁰. Studies⁵

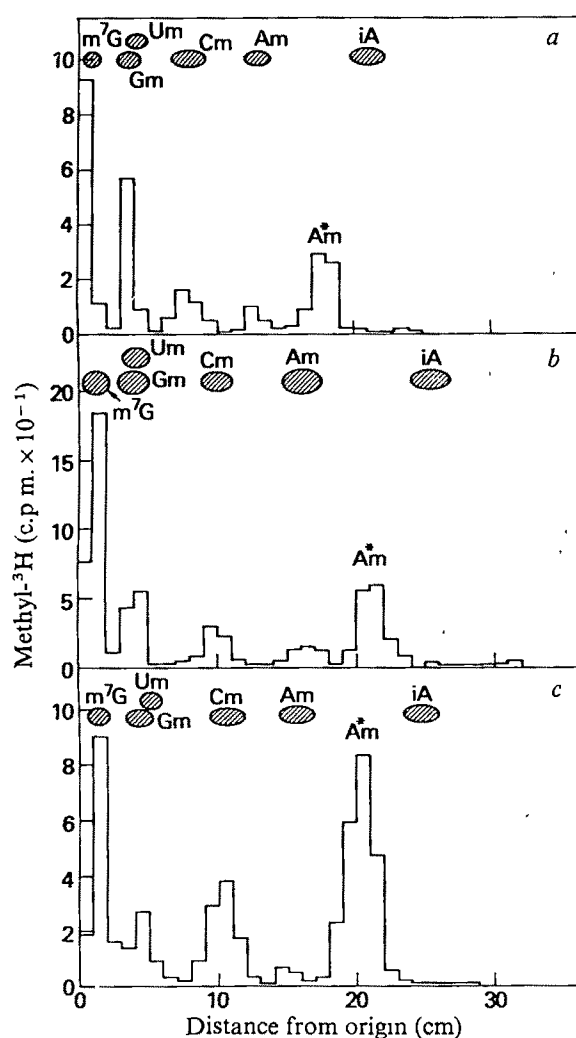


Fig. 1 Paper chromatography of methylated ribonucleosides from 5'-terminal oligonucleotides of: *a*, HeLa cell mRNA; *b*, mouse L cell mRNA; and *c*, adenovirus mRNA. Cells were labelled for 4 h with methyl-³H methionine, and polyadenylated mRNA was isolated from the cytoplasm by poly(U)-Sephadex chromatography as previously described⁵. Polyadenylated mRNA from the cytoplasm of adenovirus-infected HeLa cells was also selected by hybridisation to adenovirus DNA (B.M. and F. Kocot, unpublished). After hydrolysis of HeLa and L cell mRNAs with RNase T2, T1 and A and adenovirus mRNA with 0.4 N KOH the 5'-terminal oligonucleotides were isolated by DEAE-cellulose chromatography as the –5 to –6 charge peak⁶. The desalted 5'-terminal oligonucleotides were then digested to their constituent ribonucleosides by a combination of venom phosphodiesterase and alkaline phosphatase. The digest was analysed by ascending paper chromatography with solvent containing *n*-butanol–concentrated NH₃–H₂O (86:5:14).

of the 5'-terminal sequences of HeLa cell mRNAs revealed a methylated nucleoside that chromatographed slightly ahead of 2'-O-methyladenosine (Am). We have now identified this novel nucleoside and found that it is common to mRNAs of human and mouse cells as well as at least one virus.

The 5'-terminal oligonucleotides obtained by alkali or RNase digestion of HeLa cell mRNAs have the structures m⁷G(5')ppp(5')NmpNp and m⁷G(5')ppp(5')NmpNmpNp and elute from DEAE-cellulose columns with net charges of –5 to –6 (refs 5 and 7). Figure 1 shows a nucleoside analysis of the 5'-terminal oligonucleotides from methyl-³H-methionine-labelled mRNAs of HeLa cells, mouse L cells and adenovirus grown in HeLa cells. In addition to 7-methylguanosine (m⁷G) and the four common 2'-O-methylribonucleosides (Gm, Um, Cm and Am), a major component of each mRNA chromatographed between Am and N⁶-isopentenyladenosine (iA). The unusual nucleoside was designated A*m and tentatively considered to be a derivative of adenosine since it comigrated on electrophoresis with adenosine and Am in 1 M formic acid (Fig. 2). A*m was further shown to lack *cis*-hydroxyls by borate electrophoresis at pH 9 (ref. 5) and therefore seemed to be a derivative of Am with an additional base modification. The following experiment was designed to test whether the base was methylated and if so to identify it.

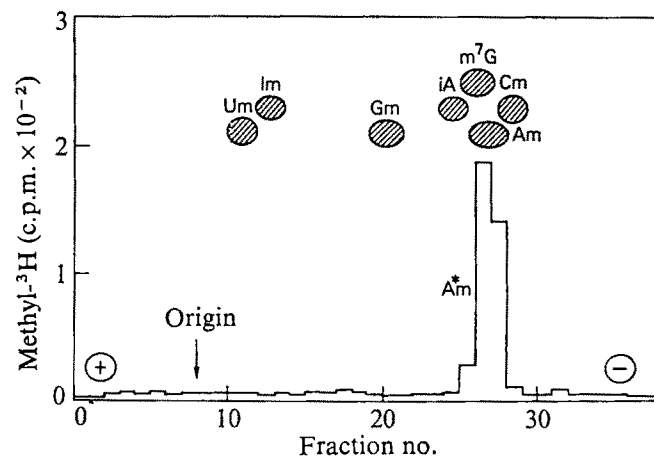


Fig. 2 Thin-layer electrophoresis of A*m. Methyl-³H-labelled A*m was isolated by paper chromatography as in Fig. 1a and analysed by electrophoresis on a thin layer of cellulose in 1 M formic acid at 800 V for 70 min.

The 5'-terminal oligonucleotides of HeLa cell mRNA were isolated and digested with P₁ nuclease (an enzyme that hydrolyses RNA to 5'-nucleotides and is not inhibited by 2'-O-methylribonucleosides¹¹) and nucleotide pyrophosphatase (an enzyme that cleaves m⁷GpppNm to pm⁷G, pNm and Pi²) to liberate methyl-labelled pA*m. The latter comigrated with pA on paper electrophoresis at pH 3.5, further suggesting that the novel compound is a derivative of adenosine. The isolated pA*m along with a small amount of contaminating pAm was then treated with 1 N HCl at 100 °C to liberate the free base. Slightly more than half of the methyl-labelled material co-chromatographed with N⁶-methyladenine (m⁶Ade) while the remainder, presumably the degradation product of 2'-O-methylribose phosphate, stayed at the origin (Fig. 3a). Since no alkaline step was used in this procedure it is unlikely that m⁶Ade was derived by rearrangement of m¹Ade. Some apparent loss of the 2'-O-methylribose derivative resulted from the lability of the O-methyl bond in 1 N HCl at 100 °C. As a control experiment, methyl-labelled pAm was isolated from the 5'-terminus of vaccinia virus mRNA¹ and treated similarly. The methyl-labelled material remained at the origin and none chromatographed as m⁶Ade (Fig. 3b).

Further evidence that the modified base of A*m is m⁶Ade was obtained by labelling HeLa cell mRNA with 2,8-³H-adenosine. Combined P₁ nuclease and alkaline phosphatase digestion followed by adsorption of the enzyme-resistant

material to a DEAE-cellulose column was used to separate the 5'-terminal oligonucleotide containing A*m from all but a small amount of the thousandfold or greater excess of internal adenosine residues. Nucleoside analysis indicated incorporation of radioactive isotope into A*m (Fig. 4a). The purified 2,8-³H-A*m was then treated with 1 N HCl at 100 °C and the labelled base released was identified as m⁶Ade in three different chromatographic systems, one of which is shown in Fig. 4b.

The sugar moiety of A*m was also characterised by cleaving methyl-³H-A*m with spleen purine nucleoside phosphorylase (Sigma) in the presence of phosphate. The labelled product cochromatographed, on a thin-layer cellulose sheet in ethyl acetate, isopropanol, 7.5 M NH₄OH, *n*-butanol (3:2:2:1), with 2-O-methylribose-1-phosphate derived from authentic Am (not shown). In the latter experiment radioactivity was lost from the methylated base because of the deaminase activity associated with nucleoside phosphorylase¹².

We conclude that the novel nucleoside found in HeLa cell, L cell and adenovirus mRNAs is N⁶,O^{2'}-dimethyladenosine (m⁶Am). Furthermore, m⁷G(5')ppp(5')m⁶Am is the major 5'-terminal sequence of the type m⁷G(5')ppp(5')Nm in both HeLa cell mRNAs, where it comprises about 20–30% of the total (C.-M.W., A.G. and B.M., unpublished), and adenovirus mRNAs, where it comprises 80% of the total (B.M. and F. Koczot, unpublished). In addition both Adams and Cory⁶ studying mouse myeloma cell mRNA, and Moyer *et al.*¹³ studying vesicular stomatitis virus mRNA synthesised *in vivo* noted an unidentified nucleoside adjacent to m⁷G which they suspected was a derivative of Am with a modified base. By contrast m⁶Am is definitely not present in either vaccinia virus¹ or vesicular stomatitis virus⁴ mRNAs synthesised *in vitro* by virion-associated enzymes, suggesting that a cellular methyltransferase is used. This methyltransferase must be quite specific since m⁶Am has not been identified in either tRNA or rRNA. A recent report¹⁴, however, indicates that an internal

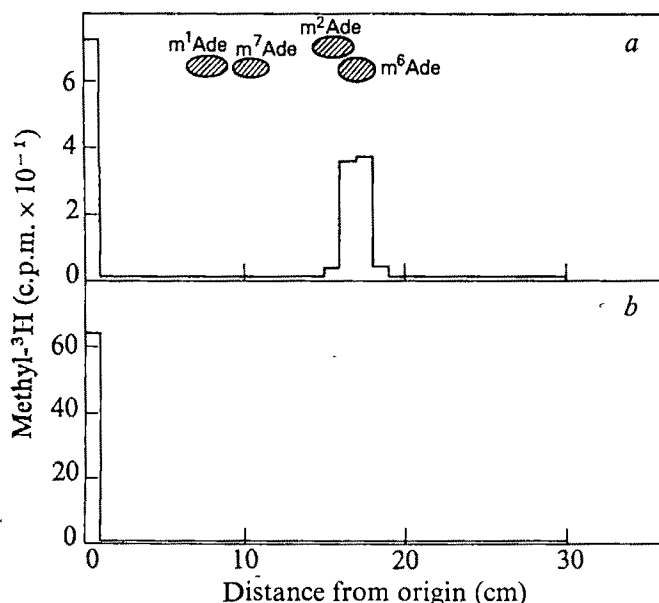


Fig. 3 Identification of methylated base. *a*, Methyl-³H-labelled 5'-terminal oligonucleotides of HeLa cell mRNA were isolated as in Fig. 1a and successively digested with 100 µg of nuclease P₁ in 200 µl of 10 mM sodium acetate; pH 6, at 37 °C for 1 h and 0.2 U nucleotide pyrophosphatase in 20 mM Tris-HCl, pH 7.6, 1 mM MgCl₂ at 37 °C for 30 min. The ribonucleotides were separated by electrophoresis on a 40 cm sheet of Whatman 3 MM paper in 50 mM ammonium formate, pH 3.5, 1 mM EDTA at 1,900 V for 3 h. The fractions corresponding to adenylic acid marker and containing pA*m were pooled. pA*m was heated with 1 N HCl at 100 °C for 30 min and the product was analysed by ascending paper chromatography with solvent containing *n*-butanol-concentrated NH₃-H₂O (86:5:14). *b*, Methyl-³H-labelled pAm isolated from vaccinia virus mRNA¹ was treated with 1 N HCl and similarly analysed.

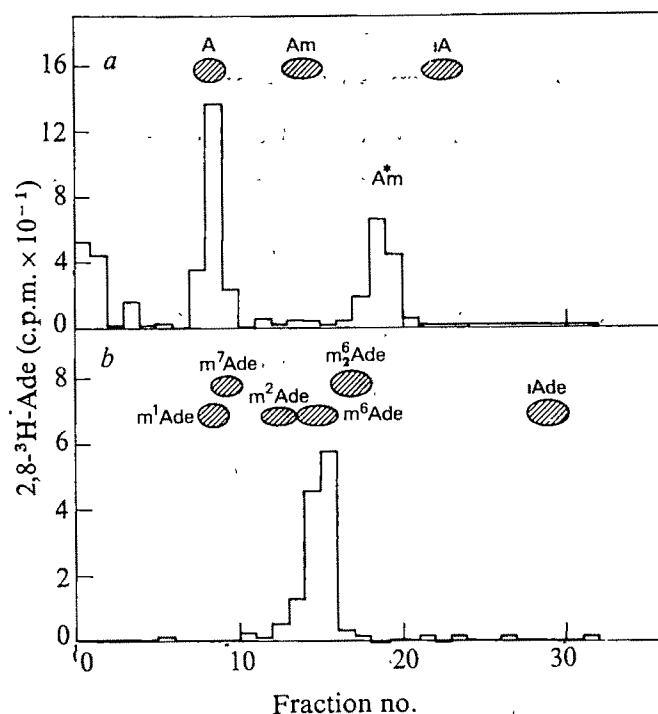


Fig. 4 Incorporation of 2,8-³H-adenine into N⁶-methyladenine moiety of A*m. *a*, HeLa cells (4 × 10⁸) were incubated in 1 l of medium containing 20 µM guanosine and 10 mCi (40.8 Ci mmol⁻¹) of 2,8-³H-adenosine for 24 h at 37 °C. The cytoplasmic polyadenylated mRNA was isolated as in Fig. 1 and then digested with nuclease P₁ (50 µg per 100 µl) in 10 mM sodium acetate, pH 6, at 37 °C for 1 h and then with alkaline phosphatase (10 µg per 100 µl) in 50 mM Tris-HCl, pH 8.5, 5 mM MgCl₂ at 37 °C for 2 h. The diluted digest was applied to a 5 ml DEAE-cellulose column equilibrated with 25 mM ammonium acetate and then extensively washed with 25 mM ammonium acetate. The labelled m⁷G(5')ppp(5')Nm was eluted with 1 M ammonium acetate, lyophilised and digested with venom phosphodiesterase and alkaline phosphatase. The nucleosides were analysed by ascending paper chromatography with solvent containing *n*-butanol-concentrated-NH₃-H₂O (86:5:14). *b*, Identification of 2,8-³H-N⁶-methyladenine. Labelled A*m isolated by paper chromatography as indicated above (*a*) was treated with 1 N HCl at 100 °C for 30 min and analysed by thin-layer chromatography with solvent containing isopropanol-concentrated HCl-H₂O (680:144:170).

m⁶Am residue is present in a low molecular weight nuclear RNA. It is possible that m⁶Am has a role in protein synthesis since in mRNAs it lies adjacent to the m⁷G residue that is required for efficient translation of certain mRNAs *in vitro*¹⁰.

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Two distinct mechanisms of synthesis of DNA fragments on colicin E1 plasmid DNA

REPLICATION of closed-circular colicin E1 plasmid (Col E1) DNA can be initiated and completed in extracts of *Escherichia coli*^{1,2}. The major products of *in vitro* replication are completely replicated molecules (23S) and a unique type of early replicative intermediate (26S) containing a newly synthesised DNA fragment(s) in a small replication loop. The fragment has an average length of approximately one fifteenth of the unit length of the plasmid molecule and has a sedimentation constant of approximately 6S^{1,3}. The replicated region of the intermediate consists of either one double-stranded branch and one single-stranded branch (type I) or two double-stranded branches (type II)^{2,4}. These intermediates accumulate in a reaction mixture containing 10% glycerol^{2,3}. Synthesis of the intermediates is inhibited by rifampicin^{1,3} but most of the intermediates can complete replication in the presence of rifampicin³. Rifampicin is also known to inhibit *in vivo* replication of Col E1 DNA⁵. We have studied the synthesis and fate of 6S DNA fragments formed on the parental heavy (H) strands and those formed on the parental light (L) strands (defined by CsCl density gradient centrifugation in the presence of poly(U,G)) of early replicative intermediates. The results show that the first synthesis of a DNA fragment is initiated at a specific site on the H strand and depends on the function of

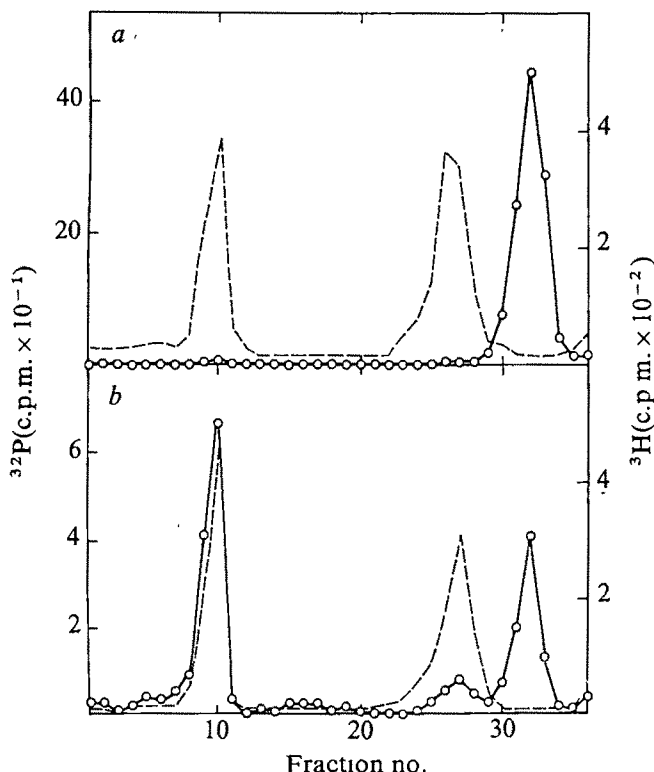


Fig. 1 Alkaline sucrose gradient centrifugation patterns of ³²P-labelled early replicative intermediates before (a) and after incubation in a cell extract with rifampicin (b). A portion of sample C (unheated) in Table 1 was centrifuged in an alkaline sucrose gradient (5–20%) at 45,000 r.p.m. for 2 h at 5 °C in a Beckman SW 50.1 rotor¹. Fractions were collected from the tube bottom. The pattern of distribution of radioactivity is shown in (a). Another portion of sample C (unheated) was incubated for 90 min at 30 °C in the standard reaction mixture¹ containing an extract of YS1 cells and rifampicin (10 µg ml⁻¹) without glycerol and spermidine. DNA was extracted and similarly centrifuged (b). The reference DNAs were, from the left, ³H-labelled collapsed closed-circular Col E1 DNA and circular and linear DNA of the unit length. —, ³²P; ---, ³H.

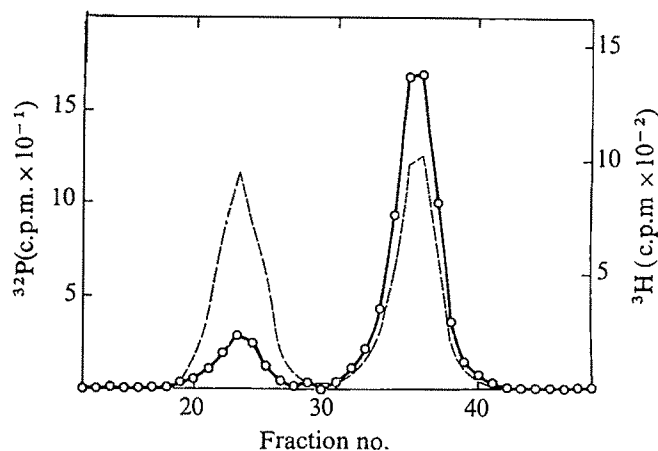


Fig. 2 Distribution of radioactivity in strands of completely replicated molecules derived from early replicative intermediates with ³²P-labelled 6S DNA. The DNA formed by incubation of sample C as described in the legend to Fig. 1b was centrifuged after heating at 90 °C for 90 s in neutral sucrose gradient (5–20%) at 45,000 r.p.m. for 150 min at 10 °C in a SW 50.1 rotor¹. Completely replicated closed-circular molecules (23S) thus isolated were converted to the linear form by treating with endonuclease *EcoRI*⁴. The linear molecules were denatured and centrifuged in a CsCl density gradient containing poly(U,G) and fractionated as described⁶. The reference DNAs were, from the left, H strands and L strands of Col E1 DNA. —, ³²P; ---, ³H

E. coli DNA-dependent RNA polymerase. Subsequent synthesis of the DNA fragment on the L strand does not involve the RNA polymerase.

To examine whether or not the strand in the type I intermediates was specifically synthesised on one of the parental strands, early replicative intermediates with 6S fragments labelled, *in vitro*, with α-³²P-dTMP were prepared (Table 1). Samples A and B were made after incubation for 10 or 45 min at 30 °C of a reaction mixture with a freshly prepared cell extract; sample C was made from a reaction mixture incubated for 45 min with a cell extract stored for 1 week at –20 °C. After purification of ³²P-labelled replicative intermediates³, labelled 6S DNA was separated from the parental strands by heating at 90 °C for 90 s (ref. 3) and then annealed with separate H and L strands of Col E1 DNA fixed on membrane filters. Table 1 shows that in all cases more labelled 6S fragments were hybridised to H strands than L strands. Synthesis of two types of 6S fragments was more asymmetrical for the molecules labelled for 10 min than for 45 min. It was also more asymmetrical for the molecules synthesised with the stored extract than with the fresh extract. In sample B, 33% of 6S fragments was hybridisable to L strands, while about 40% of such a preparation of intermediates was type II as examined electron microscopically². These results show that most type I intermediates had a 6S L fragment.

Formation of early replicative intermediates is sensitive to rifampicin, but most of the intermediates formed in a cell extract can complete replication during continued incubation after addition of rifampicin³. These results indicate either that no further products of the rifampicin-sensitive reaction were necessary for completion of replication of the intermediates, or that products formed before addition of rifampicin could be used for the replication that took place after addition. The validity of these interpretations was tested by examining replication of exogenously added early replicative intermediates in the presence of rifampicin. The preparation of the intermediates (sample C of Table 1) used for this purpose was practically free from labelled, completely replicated molecules as shown by alkaline sucrose density gradient centrifugation of the preparation (Fig. 1a). By contrast, the labelled DNA extracted after the incubation of the intermediates in the presence of rifampicin contained approximately 55% collapsed closed-circular molecules and 10% unit-length single strands (Fig. 1b). Completely replicated molecules were formed from

the early replicative intermediates also in the presence of antibody against *E. coli* RNA polymerase which inhibited formation of the intermediates (data not shown). To test whether type I intermediates replicate in the presence of rifampicin, the distribution of radioactivity between H and L strands of completely replicated molecules derived from the intermediates was examined. After conversion of the molecules to the linear form by treating with endonuclease *EcoRI*⁴, the single strands of the molecules were centrifuged in a CsCl density gradient containing poly(U,G)⁶. The labelled DNA was distributed in L and H strands at the ratio of approximately 6:1 (Fig. 2). If only the type II intermediates completed replication or if labelled DNA was degraded and labelled material was reincorporated into closed-circular molecules, the radioactivity would have been distributed almost equally between the complementary strands of the molecules. The unequal distribution of radioactivity between the complementary strands indicates that the type I intermediates containing a 6S fragment on the parental H strand were converted to completely replicated molecules, in the presence of rifampicin. These results indicate that the product synthesised in the extract by *E. coli* RNA polymerase is not necessary for completion of replication of the intermediates.

Table 1 Strand-specificity of 6S DNA in early replicative intermediates

DNA fixed on filter ³² P-DNA	³² P-DNA hybridised (c.p.m.)		³² P-DNA applied (c.p.m.)	
	H strands	L strands	Calf thymus (blank)	
Samples				
A, heated	209 (76%)	68 (24%)	2	884
B, heated	538 (67%)	278 (33%)	20	2,650
C, heated	790 (82%)	170 (18%)	10	2,907
Controls				
H strands	26 (4%)	467 (96%)	7	1,370
L strands	447 (88%)	61 (12%)	7	1,267

Early replicative intermediates labelled with α -³²P-dTMP were prepared by incubating Col E1 DNA (10 μ g ml⁻¹) and an extract of *E. coli* YSI cells (100 μ l) in the standard reaction mixture (300 μ l) containing α -³²P-dTTP (0.5 Ci mmol⁻¹ for samples A and B; 2 Ci mmol⁻¹ for sample C), 10% glycerol and 2 mM spermidine². A freshly prepared extract was used for preparation of samples A and B. An extract which had been frozen and thawed twice during storage for a week at -20°C was used to prepare sample C. The reaction mixture was incubated for 10 min (for sample A) or 45 min (for samples B and C) at 30°C. DNA was extracted and early replicative intermediates (26 S) were isolated by neutral sucrose density gradient centrifugation^{2,3}. A portion of these samples was heated at 90°C for 90 s to separate 6S fragments from the parental strands¹. Since the amount of contaminating labelled completely replicated molecules was negligible (Fig. 1a and ref. 2), the heated samples were used to hybridise to separate strands of Col E1 DNA without further purification. The two strands of Col E1 DNA that had been treated with endonuclease *EcoRI* and denatured were isolated by CsCl density gradient centrifugation in the presence of poly(U,G)⁶. Col E1 DNA labelled with ³H-thymine (1 mCi mmol⁻¹)⁴ or ³²P-H₂PO₄ (100 mCi mmol⁻¹)² was used to prepare the separate strands. ³H-labelled separate strands or denatured calf thymus DNA (0.3 μ g each) was fixed on a membrane filter⁷. Hybridisation experiments were carried out essentially as described⁷ except that three filters each of which was loaded with the H strands, L strands or calf thymus DNA were placed together in a hybridisation mixture (1 ml). After incubation for 18 h at 64°C, the filters were washed and radioactivity was measured. The ³²P-labelled separate strands were used to test cross contamination of ³H-labelled separate strands fixed on filters. The ³²P-labelled separate strands were purer than the ³H-labelled separate strands because the concentration of ³²P-labelled strands (0.01 μ g ml⁻¹) used in the centrifugation for strand separation was much less than that of ³H-labelled strands (2 μ g ml⁻¹) used. The values in parentheses are the fractions of labelled DNA which hybridised to separate strands in the total hybridised DNA (the blank value was subtracted). Based on the results in row 4 and 5, it may be argued that the fraction of 6S H DNA in the total 6S DNA may be slightly overestimated.

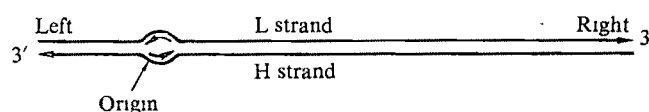


Fig. 3 Polarity of the strands of an early replicative intermediate (*EcoRI* cleaved) and the site of origin of replication.

Since most type I intermediates had a 6S L fragment and these molecules can complete replication, the site of initiation of replication of a 6S L fragment is the origin of replication of most of the molecules. The site is the 5' end of the fragment because DNA polymerase I, which extends a polydeoxyribonucleotide chain in the 5' to 3' direction⁸, is involved in the synthesis of the fragments (my manuscript in preparation). The polarity of a 6S L fragment can be deduced from that of Col E1 DNA strands, which was determined previously⁷. The 3' end of the H strand in the linear molecule created by cleavage of a closed-circular Col E1 DNA molecule by *EcoRI* is located closer to the region of early replication than the 5' end of the strand. The end of the linear molecule with the 3' end of the H strand corresponds to the left end of the molecule previously defined⁴. Therefore the 5' end of the 6S L fragment is located at the left branch point (approximately 17% of the molecular length from the *EcoRI* site^{2,4}) in the intermediate as illustrated in Fig. 3. It should be noted that unidirectional expansion of a replication loop, as was found in electron microscopic studies^{4,9,10}, does not mean that initiation of replication occurs from the fixed branch point since the method, in principle, does not reveal the site of initiation of replication and the direction of replication within the smallest replication loop observed.

Different mechanisms of initiation of synthesis of DNA strands on single-stranded phage DNAs have been reported. Initiation of synthesis of the complementary strand on M13 phage DNA is primed by *E. coli* RNA polymerase which presumably recognises a double-stranded region of the DNA¹¹. On the other hand, synthesis on ϕ X174 phage DNA and G4 phage DNA does not involve the RNA polymerase^{12,13}. In the replication of Col E1 DNA, initiation of synthesis of 6S L fragments in early replicative intermediates is completely blocked by rifampicin, while synthesis of 6S H fragments as well as further replication of the intermediates is not inhibited by rifampicin. Purified *E. coli* RNA polymerase can carry out a reaction that is equivalent to the rifampicin-sensitive reaction and is probably involved in synthesis of the primer for 6S L fragments (J. T. and T. Kakefuda, unpublished). Thus the mechanism of synthesis of the first fragment from a specific origin of replication is different from that of the fragments subsequently formed. Utilisation of the two distinct mechanisms may be advantageous by reducing the chance of aberrant initiation of replication of the molecule.

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matters arising

Empirical formulae for Student's function

THE empirical formula devised by Dawson¹ for the calculation of the theoretical values of the Student's t function at any level of probability is a useful addition for many statistical programs. I have found that the following empirical formulae provide a simple method for calculating t at various defined probability levels, P :

$$P = 0.10; t = 1.64485 + 1/(0.648n - 0.534)$$

$$P = 0.05; t = 1.95996 + 1/(0.413n - 0.423)$$

$$P = 0.01; t = 2.57582 + 1/(0.193n - 0.273)$$

where n is the number of degrees of freedom. With three or more degrees of freedom these formulae give t accurate to 0.06%, 0.1% and 0.5%, respectively. A computer program using these formulae would require only the addition of the values of t for one and two degrees of freedom. These formulae are not only more accurate than Dawson's, but are also more suitable for hand calculators.

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¹ Dawson, F. H., *Nature*, 256, 148 (1975).

Cape diastrophism

IN a recent paper¹ on the Cape Fold Belt, the folded strata discussed range in age from late Ordovician² to late Permian³. Until the early Permian these strata were derived from the north^{4,5}. The ensuing palaeoslope reversal⁶ marks the lower limit of the Cape diastrophism, and transportation of sediment from the west and south can be related to the two fold trends subsequently developed⁷. Karroo dolerites are excluded from the fold belt, and their upper age limit⁸ defines the end of the diastrophism. From the Ordovician to the early Jurassic there are very real Gondwana links: for example, the Malvinokaffric fauna⁹, the distribution of the Permo-Carboniferous glaciation¹⁰, of *Mesosaurus*¹¹, and of *Lystrosaurus*¹². The diastrophism was terminated by major faulting, and earliest marine sediments¹³ occur in the mid-Jurassic. This was the forerunner of the spreading of the South Atlantic, which commenced at this latitude about 130 Myr ago¹⁴. Before that the Falkland Plateau

lay immediately south of the Agulhas Bank¹⁵.

de Beer and his coauthors proposed¹ that an oceanic plate underthrust Gondwanaland coincident with the present southern limit of Africa, and that the Cape Fold Belt resulted from an ensuing continent-continent collision directed from the south. The data given here cannot be reconciled with this suggestion for five reasons.

First, slow shelf subsidence characterised the area for almost 200 Myr before folding. Second, there are two fold trends, one parallel to the proposed direction of collision. Third, the Agulhas Bank is considered to be floored by similar material to that present onshore¹⁶, and presents no evidence of a major suture. Fourth, the impressive similarity of the Emsian faunas of South Africa and the Falkland Islands¹⁷ suggests that the position of the latter in Devonian times was the same as in the Jurassic. Finally the concept of a plate underthrusting Gondwanaland from the south implies that Gondwanic continents such as South America and Antarctica were not in the pre-Mesozoic positions commonly ascribed to them and consequently, that the collision would involve a continent lacking Gondwana affinities. However the Cape diastrophism may have formed, a plate tectonic model is not well served by failure to consider such material evidence.

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DE BEER ET AL. REPLY—Truswell's comments¹ deal mostly with matters outside the scope of our original letter², which was intended to consider geophysical anomalies near the Cape Fold Belt and, if possible, to relate these to proposed geological models. We agree that the model suggested in the paper is simplistic and could be misinterpreted.

Although the folded strata range in age from Upper Ordovician³ to Upper Permian⁴, the main phase of folding was in the Triassic⁴. The less severe, north-south trending Cedarberg folding occurred in pre-Dwyka times⁴. The first signs of a southern land mass⁵ and pre-Dwyka folding in the east-west trending fold belt⁴ are observable in Witteberg (Lower Carboniferous) strata. This southern land mass was not simply an uplifted part of the continental craton, but an island arc type of orogenic belt, possibly related to a subduction zone south of the present coastline^{6,7}.

An important point is that the Cape Fold Belt represents only the northern edge of the more extensive Samfrau geosyncline of Gondwanaland^{8,9}. The orogenic belt and igneous rocks related to the inferred subduction zone are thus not in South Africa but lie in Patagonia and western Antarctica^{7,9,10}.

Truswell's objections to our model can be covered point by point.

First, the folding in the southern Cape occurred during Lower Carboniferous times, which means that shelf subsidence lasted only about 100 Myr before the start of any orogenesis related to subduction. Second, the degree and type of deformation accompanying continental collision (or continent-island arc collision) depend on the shapes of the continents and the morphology of the trench system, or systems, involved (see, for example, ref. 11). A fold system with only two fold trends of different ages will require a relatively simple model. The relevant geological and geophysical evidence from South America and Antarctica must be included in such a model. Third, this indicates that the

suture will be further south. Fourth, geophysical evidence strongly suggests an Upper Triassic–Lower Jurassic Gondwana model in which the Falkland Plateau as part of the South American plate borders the African plate^{12–14}. This model is consistent with Truswell's considerations and with the hypothesis that the Falkland islands were to the north of the inferred subduction zone. The position of the subduction zone with respect to Patagonia is unclear. If it was situated to the east of Patagonia, the suture could lie in the basement structures in the continental edge and in the Malvinas Basin¹⁵. The suture could then cross the continent in the Upper Permian Patagonian Massif⁸ or the Middle Triassic Desado Massif¹⁶. Geological evidence, however, strongly supports the existence of a subduction zone that bordered the western rim of Patagonia from Devonian to Triassic times^{7,17}.

Fifth, from the Gondwana model it is clear that parts of Patagonia and the Antarctic Peninsula formed the southern land mass that approached southern Africa during Palaeozoic times in a process peripheral to the main Gondwana land mass. Even this accreted land mass should show affinities with Gondwanaland from at least Lower Carboniferous times.

This first order extension of our very elementary model inferring a continental collision origin for the Cape Fold Belt shows that the constraints proposed by Truswell can indeed be reconciled with a continent–continent (or at least a continent–island arc) collision south of Africa.

Our explanation of the origin of the Beattie magnetic anomalies is more contentious and should perhaps be related to processes causing the accretion of the present-day basement in the Karroo and further south rather than to processes causing the formation of the fold belt.

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Chocolate, β -phenethylamine and migraine re-examined

ON the basis of an industrial analytical report indicating that chocolate might contain as much as 3 mg of β -phenethylamine (PEA) per 2-ounce bar, Sandler *et al.*¹ administered 3 mg of this amine to each of 36 chocolate-sensitive migraine-prone subjects and found that 18 suffered attacks within 12 h, as opposed to six attacks with placebo.

zene-ethyl acetate (9:1). A portion of the eluted radioactive zone was counted and a smaller portion was injected on to a column containing 3% Poly A-103 on Gas Chrom Q (F and M Model 400, fitted with Hewlett-Packard ⁶³Ni electron capture detector). With the use of external standards prepared from crystalline N-heptafluorobutyryl-PEA and an estimate of losses provided by recovered ¹⁴C, a simple calculation provided the native PEA level.

As Table 1 shows, the highest level, 780 μ g per 2 ounce, was found in the unsweetened variety. Semi-sweet forms contained intermediate amounts and milk chocolate was lowest in PEA. The decline may be related to the amount of time the raw chocolate is subjected to conching, a mellowing process during manufacture which includes rolling and heating. PEA, a relatively volatile amine, could escape during this step. The modest rise in PEA after relatively severe hydrolysis indicates that presumed conjugates do not contribute substantially to the total potential PEA pool.

Table 1 PEA levels in various brands of chocolate

Brand	Country	Type*	μ g PEA-HCl per 2 ounce		Recovery of PEA-HCl added to 1 g chocolate		
			First analysis	Second analysis	Added (μ g)	Native level (μ g)	Total recovered (μ g)
A	England	Milk	38	41	—	—	—
B	USA	Mild sweet	285	315	1.0	5.0	6.2
		Milk	78	85	50.0	1.4	57.0
C	USA	Mildly sweet	330	—	—	—	—
		Milk	21	—	—	—	—
D	USA	Semi-sweet	360	390	1.0	6.4	8.2
		Unsweetened	780	978†	20.0	13.8	35.7
E	Switzerland	Milk	86	—	—	—	—
		Bittersweet	260	—	—	—	—
F	Switzerland	Milk	103	—	—	—	—
		Bittersweet	340	—	—	—	—

*Manufacturer's description.

†Sample subjected to 100 °C hydrolysis in 6 M HCl for 0.5 h.

Having previously developed a sensitive procedure for the analysis of PEA in urine², we applied it with minor modifications to the analysis of one US brand of milk chocolate. We found only 21 μ g per 2-ounce bar, or about 1/150 of that quoted above. Various brands and types of chocolate were then examined.

In the method, 1-¹⁴CPEA, 8.86 mCi mmol⁻¹, was used as an internal standard. About 10⁵ d.p.m. were added to 1–2 g chocolate melted in 5 ml water. With some samples, 1–50 μ g of unlabelled PEA was also added in tests to demonstrate recovery. Briefly (see ref. 2 for details), bases were extracted with chloroform. After concentration, the residue was applied to a silica gel plate and developed in ethylacetate–methanol–diethylamine (9:1:1). The labelled area, eluted and derivatised with heptafluorobutyric anhydride, was chromatographed in ben-

These data suggest that chocolate-induced migrainous attacks are either not related to PEA-induced headaches, or that patients suffering from migraine are sensitive to very low levels of PEA.

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reviews

THE first volume of this textbook, dealing with the principles of the subject, appeared in 1962 and reached a second edition in 1968. Good of its kind, it necessarily follows fairly conventional lines. The second volume aims at global history in a way that has never previously been attempted. It was about three-quarters complete when H. H. Read died in 1970, in his 80th year; Professor Janet Watson is to be congratulated on bringing the eagerly-awaited work to fruition.

The method adopted is to integrate the broad record of stratigraphy of rocks formed at or near the Earth's surface with structural, magmatic and metamorphic events which occurred at depth within the crust. The evidence comes fundamentally from geological mapping at the surface, with data on the third dimensions derived from valleys, mines, boreholes and the indirect probing of geophysics. The process has been carried to a very variable degree in different parts of the world, but there is no doubt that the time is ripe for synthesis on the scale attempted here. Moreover, the classical method of time-assessment from fossils has now been carried far back into the Cryptozoic, and the newer method of radiometric dating gives a valuable control over Cryptozoic and Phanerozoic alike, once its limitations have been appreciated. The authors point out, however, that they have had to place severe limitations upon the amount of stratigraphic (and, therefore, palaeontological) detail given. The framework is, in fact, not stratigraphy but tectonics and this follows very suitably from the last chapter of Volume 1 ("A pattern of earth history") which drew a sharp distinction between stable regions of the crust and belts of mobility. The history of the

New geological framework

Introduction to Geology. Vol. 2: Earth History. By H. H. Read and Janet Watson. Part 1: *Early Stages of Earth History*. Pp. xii+221. £5.95 board; £2.95 paper. Part 2: *Later Stages of Earth History*. Pp. xii+371. £6.95 board; £3.95 paper. (Macmillan: London and Basingstoke; distributed in the USA by Halsted Press; May 1975.)

mobile belts is adopted as the framework for Volume 2, though the cratons of Precambrian time, the interior lowlands, epicontinental basins and forelands are by no means forgotten.

Volume 2 is issued in two parts in order to make possible the publication of the paperback edition. Part I deals with the first 2.9×10^9 years, covering Precambrian events up to the later Proterozoic. As far as I know, this is the first time that a global synthesis of the Precambrian, involving all the continents including Antarctica has been attempted. For each continent, an outline of the tectonic pattern is first given; then the stages by which the rocks older than 900 Myr have been formed is set forth. A concept of a series of cycles emerges; the ending of a cycle brings stability to tracts that were formerly mobile, contributing to the growth of cratons; but the initiation of a new cycle may affect tracts that were formerly stable.

Many interesting problems for future solution come directly from this synthesis.

Though it seems unlikely that a stratigraphic classification like that adopted throughout the world for the Phanerozoic will ever become feasible for the ancient rocks, some correlations certainly begin to emerge, as Figure 10.1 shows in the book. Indeed Part I will make good reading for those involved in the Precambrian priority area of the International Geological Correlation Programme of UNESCO/IUGS.

Part II commences with a chapter on new themes in Earth history which provides a convenient way of dealing with continental drift and palaeoclimatic change. The method used in Part I is then readopted and the framework again established on mobile belts, but belts which will be much more familiar to most readers than those described in Part I: the Caledonian, Hercynian-Uralide, Alpine-Himalayan and Cordilleran. The interior platforms of little distorted cover-rocks resting on Precambrian or later basement receive due attention, and the break up of the great ancient southern continent of Gondwanaland is described. This leads on to the current concepts of global tectonics, the opening of the Atlantic and Indian oceans, and the outward circumferential push around the Pacific.

Even if the authors have had to scale down the over abundant geological detail, they have succeeded in following those aspects of geological process that can be traced throughout the full span of Earth history, at the same time pointing a finger at a few, like banded ironstone formation or the detrital transport of uraninite, that long ago ceased for ever. Surprisingly in this historical essay, the useful rocks and minerals are not forgotten, but appear in their due genetic positions.

Kingsley Dunham

TRANSPORT of peptides across biological membranes, first discovered in microorganisms, is now recognised to be of major significance in the intestinal absorption of amino-N, yet little is known of other roles of peptide transport.

The variety of peptides— $20^2=400$ dipeptides alone—and the range of their biological activities is astonishing. Some are hormones, others vasoactive. There are peptides called 'smugglers' that carry otherwise impermeant molecules piggyback across membranes. Peptides contribute sig-

Peptide transport

Peptide Transport in Protein Nutrition. (North-Holland Research Monographs, Frontiers of Biology, Volume 37.) Edited by D. M. Matthews and J. W. Payne. Pp. xxii+503. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1975.) Dfl. 130; \$54.25.

nificantly to the flavour of foodstuffs. Some taste extremely sweet, others containing acidic and neutral amino

acids taste sour but (see page 55) Mrs Beeton's recipe for tasty beef tea produces a satisfactory mixture.

The Editors and their eight colleagues have succeeded in delving into diverse disciplines to produce a generally well-balanced account of knowledge of peptides, with the emphasis on nutrition.

This is an expensive book (10 pages = \$1.00), but an essential acquisition for libraries. It will be read with profit by biochemists, clinicians, nutritional scientists and food technologists.

Dennis Parsons

Guide to the Marine Stations of the North Atlantic and European Waters. Part I. Northern Europe and the East Atlantic Coast. Compiled by J. E. Webb. Pp. 263+34 figs. (The Royal Society; London, 1974.) £2.50 (UK); £2.60 (overseas).

THIS guide, compiled by Professor J. E. Webb, is the first of a new series of handbooks which will be extended in further parts to cover marine stations of the Mediterranean, the Atlantic seaboard of USA and Canada, and the Caribbean and Gulf of Mexico.

Part I is an invaluable source of information on the facilities offered at 33 European marine stations, including 12 within the United Kingdom and Eire. The information is concise and practical. Short descriptions are given of the main types of shores to be found in the neighbourhood of the stations; of the common animals that can be obtained without difficulty; at the time of year when they occur; of the ships, boats and vehicles that are available for field surveys; and of the types of gear at hand for the collection of specimens and data.

The number and nature of the services provided in the research rooms and laboratories of the station are listed. The availability of special apparatus, such as is often required by physiologists, is briefly but usefully noted; and an intending visitor may be guided on the nature of the apparatus, equipment and materials which a station may be able to provide. Notes on the voltage, d.c./a.c. frequency, and phase of the electricity supply at the various stations are especially useful. Excellent outline maps showing the locations, the nature of the shorelines and the approaches to stations, illustrate the guide.

The guide, as noted in the preface, is not intended to be comprehensive. Some additional information would perhaps have been useful, such as the languages commonly spoken, the technical assistance that a visitor might expect to receive and fuller notes on the characteristics of the plankton and algae. The guide is, however, in a form which lends itself to ready revision as the need arises, and in its present form it is up-to-date and informative. **J. E. Smith**

Comprehensive Virology. Edited by Heinz Fraenkel-Conrat and Robert E. Wagner. Vol. 2: Reproduction—Small and Intermediate RNA Viruses. Pp. xiv+340. \$33.60. Vol. 3: Reproduction—DNA Animal Viruses. Pp. xiv+488. \$38.50. Vol. 4: Reproduction—Large RNA Viruses. Pp. xii+347. \$34.90. (Plenum: New York and London, 1974-75.)

VOLUME I of *Comprehensive Virology* was essentially a catalogue, containing a very limited amount of descriptive material. The next four volumes, of which volumes 2, 3 and 4 are now published, are entirely different from Volume 1, and are all concerned with the ways in which viruses reproduce themselves. These are major reviews, and although one or two contributions show signs of being variations upon earlier texts by the same author(s), much of the work is new and up to date. The sections in Volume 2 by E. R. Pfefferkorn and D. Shapiro on togaviruses, and in Volume 3 by B. Roizman and D. Furlong on herpesviruses, are particularly fresh and original. Later volumes will deal with structure and assembly, and with regulation and genetics. This approach means that a virologist with an interest in one particular virus family is obliged to buy three volumes in order to cover all aspects of his particular virus. By so doing, however, he is likely to read more widely than if he confined himself to single-virus books on 'poxviruses' or 'herpesviruses', and will thus benefit himself as well as the publishers. **J. S. Porterfield**

Books brief

Introduction to Geochemistry. (Geophysics and Astrophysics Monographs, vol 10.) By Claude-Jean Allègre and Gil Michard. Pp. viii+142. (Reidel: Dordrecht and Boston, Massachusetts, 1974) n.p.

Handbook of Geochemistry, vol. 2, part 4. Edited by K. H. Wedepohl. Pp. vi+898. (Springer: Berlin and New York, 1974.) DM.298; \$122.20.

WERE he to judge from the title the tyro might expect *Introduction to Geochemistry* to provide a comprehensive appraisal of geochemistry at a reasonably elementary level. Instead, he will find a good introduction to certain important geochemical processes. The book confines itself essentially to element fractionation: the fractionation of major and trace elements through magmatic processes and in the hydrosphere; the fractionation of light isotopes, with particular reference to $^{18}\text{O}/^{16}\text{O}$ and $^{32}\text{S}/^{34}\text{S}$ isotopic compositions; and of radioactive isotopes. Natural processes in which chemical equilibrium is not attained are also considered.

The book is a translation, "done without amendments, editings or alterations", and, consequently, it appears as a translation, although the

sentence, 'A test of the validity of these equations can be gotten here in a simple way' conjures up some thoughts about the original French. The authors' "rule" to omit many text citations from the bibliographies is irritating.

The *Handbook of Geochemistry*, the latest part of a continuing project, upholds its high standard of content and production, and meets its publication date. The authors, editors and publishers are all to be congratulated on maintaining the impetus of the project. Chapters covering a further 24 elements (including carbon, nitrogen, oxygen, silicon, magnesium and iron) are now supplied; little is missing from another 21 chapters; and with more than three quarters of the elements completed only relatively small gaps remain. Inevitably, with publication over a six-year period, costs have escalated, but at least fairly uniformly. The price is now 14.7 cents for each page, compared with a price of 5.5 cents in 1969. The *Handbook of Geochemistry* is invaluable to earth scientists, but regrettably, it is a library purchase, and these days even some libraries may resist temptation. **Duncan Murchison**

Fast Reactions. (Oxford Chemistry Series.) By J. N. Bradley. Oxford Chemistry Series (Number 23). Pp. x+121. (Clarendon: Oxford; Oxford University Press: London, June 1975.) £3.25.

PROFESSOR Bradley has written a very concise description of fast reaction kinetic studies, emphasising throughout his book the experimental rather than the chemical aspects of the subject. The descriptions of the individual techniques are not very detailed, and the author's own interest in gaseous, rather than solution work, is very apparent. A preferable presentation would have been to split the subject into two parts of this series, one referring to gaseous the other to solution kinetics. In any case the value of the book would be greatly enhanced by the provision of many more up-to-date chemical examples, particularly for the solution kinetic techniques. The standard of production of the book does not compare favourably with others in the series: the print was blurred on many of the pages of the reviewer's copy, and the line lengths had not been justified. A number of diagrams need better description or more labelling, and there are a few text errors. The reviewer will continue to use Caldin's *Fast Reactions in Solution* (1964) and Hague's *Fast Reactions* (1971) as preferred recommended texts.

John Maher

Bonded states

Metal-to-Metal Bonded States of the Main Group Elements. By M. J. Taylor. Pp. 211. (Academic: New York and London, May 1975.) \$15.25; £5.80.

COMPOUNDS in which ligands are linked to a cluster of atoms, instead of to a single atom, have opened up much novel chemistry in recent years. Much of this has been in transition metal chemistry, but cluster compounds spread right across the periodic table, and interest in them stems from their novel compositions, the apparently low valence states of the elements, their stereochemistry and their valence-theoretical description.

Dr Taylor has explicitly restricted himself to compounds of the main group elements. In addition, he limits consideration to compounds of elements that, in the elementary state are regarded as metals. There is no real justification for doing so, and the arbitrary restriction has some illogical consequences. Thus, he has to discuss the cationic clusters formed by selenium and tellurium without proper reference to their exact analogues in sulphur chemistry, and he makes no mention at all of the anionic clusters formed by silicon and germanium. But equally mistakenly in my opinion he broadens his survey to include all compounds in which two 'metal' atoms are linked. The real interest of metal-metal bonding surely resides in the consequence of electron delocalisation within a cluster or chain of identical (or at least essentially interchangeable) atoms. The pro-

perties of, for example, the $(\text{CH}_3)_3\text{Sn}$ group, like those of the $(\text{CH}_3)_3\text{Si}$ or even the $(\text{CH}_3)_3\text{C}$ group, are those of a straightforward σ -bonding group; there is nothing special about such compounds as $(\text{CH}_3)_3\text{SnCo}(\text{Co})_4$, or $(\text{CH}_3)_6\text{Sn}_2$, to justify special attention to the 'metal-metal' bond. The amount of space devoted to compounds of this kind dilutes what should be the main interest of the book.

What the book does provide is much information on the formation of dimer and cluster species, in solutions and melts, and structural information based on vibrational spectroscopy and crystal structure. It has a thorough bibliography, heavily biased towards work of the last 15 years, including references up to 1973. This review content is its main value. For student reading, it does not bring a unifying outlook to a diverse field; for example, there is repeated terminological confusion between the oxidation states, which may often be experimentally determined, and the valence states of elements which in cluster compounds are certainly different from the oxidation states and which may be debatable. There are many typographical errors, but few will lead to confusion. Readers will note that, on the dust jacket, Academic Press have now decreed that selenium and tellurium shall be Group Vb elements.

The author states that the book is an expanded treatment of a review. One must query whether that expansion was really justified. A good review does not necessarily produce an equally good monograph. **J. S. Anderson**

Literature on water

Water and Aqueous Solutions: Introduction to a Molecular Theory. By Arieh Ben-Naim. Pp. xvi+474. (Plenum: New York and London, 1974.) \$34.90.

THE basic thesis of this book is that some sort of mixture model provides a fundamentally correct physical description of the thermodynamic properties of water and aqueous solutions and that mixture models can be made respectable or rigorous by embedding them in or deriving them from the fundamental statistical mechanical theory of fluids. The author is not particularly concerned with establishing the validity of any particular mixture model. Rather, he is concerned with the features common to all such models and with giving precise statistical mechanical definitions of the concepts used in discussing them.

The first half of the book discusses the statistical mechanics of fluids, molecular distribution functions, and the theory of solutions. The last half is concerned with liquid water, water with one simple (non-hydrogen-bonding) solute, and

water with two simple solutes. The emphasis in this second half is almost entirely on thermodynamic properties, rather than on time dependent or spectroscopic measurements. A major limitation of the book (which is a reflection of when its writing was completed) is that it contains only a brief discussion of the earliest computer simulation studies of realistic models of water. Such studies should provide the most convincing evidence for or against the usefulness of mixture models.

Many of the features of the book are of interest and of value even to the reader who does not share the author's preference for mixture models; for example, the careful discussion of those aspects of solution theory which are needed to understand experiments on aqueous solutions, the precise discussion of standard thermodynamic quantities of transfer, and a statistical mechanical definition of the concept of hydrophobic interaction. Though the book does not answer many questions it does formulate many about water and aqueous solutions in a precise way. It is a useful companion volume for the large recent review literature on water. **Hans C. Andersen**

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announcements

Award

H. C. Hottel and **H. Tabor** have jointly been awarded the 1975 **Royal Society Esso Award for the Conservation of Energy** for their work on solar energy collection.

Appointments

Sir Andrew Huxley is to be the 1976–1977 president of the British Association for the Advancement of Science.

J. S. Anderson has been appointed Honorary Professorial Fellow and **J. L. Hutchison** Research Associate to the Solid State Chemistry Group at the Edward Davies Chemical Laboratories, University College of Wales. The Science Research Council has awarded a grant to support their research.

International meetings

November 3–5, **Refuse-to-energy**, Montreux, Switzerland (Professor N. Y. Kirov, University of New South Wales, Department of Fuel Technology, PO Box 1, Kensington, NSW 2033, Australia).

November 3–6, **Advances in chromatography**, Munich, Germany (Professor A. Zlatkis, Chemistry Department, University of Houston, Texas 77004, US).

November 3–6, **High energy particle interactions**, Smolenice, Czechoslovakia (Dalibor Krupa, Institute of Physics, Slovak Academy of Sciences, Dubravska Cesta, CS-89930, Bratislava).

November 3–7, **Biological effects of low level radiation pertinent to protection of man and his environment**, Chicago, US (International Atomic Energy Agency, PO Box 590, Kartner Ring 11, A-1011, Vienna, Austria)

November 3–7, **Biomedical transducers**, Paris (Secretariat, International Conferences, c/o F.N.I.E., 16, rue de Presles, F-75740 Paris Cedex 15).

November 3–7, **CIOS congress**, Caracas, Venezuela (International Council for Scientific Management, 1 rue de Varembe, BP 20, CH-1211, Geneva, Switzerland).

November 4–8, **Molecular basis of circadian rhythms**, Berlin (Dr S. Bernhard, Dahlem Konferenzen, 1 Berlin 33, Delbruckstrasse 4c, Germany).

November 9–15, **American water sources**, New Orleans (AWRA, St Anthony Falls Hydraulic Laboratory, University of Minnesota, Mississippi River, Third Avenue, Minneapolis, Minn. 55414, US).

November 10–11, **Energy saving in chemical processes**, Milan (FAST, Piazzale R. Morandi 2, 120 Milan, Italy).

November 10–13, **Sensing environmental pollutants**, Las Vegas (B. H. Mannheimer, Department of Housing and Urban Development, Washington D.C. 20410, US)

November 16–19, **Association of earth sciences, editors annual meeting**, Hershey, Pa. (J. P. Wilshusen, Pennsylvania Geological Survey, Department of Environmental Resources, Harrisburg, Pa. 17120, US).

November 16–20, **Laboratory animal science**, Boston, Massachusetts (Mr J. J. Garvey, AALAS, 2317 W. Jefferson Street, Suite 208, Joliet, IL. 60435, US).

November 16–26, **Human environment**, Kyoto, Japan (Science Council of Japan, 22–34 Roppongi, 7 Chome, Minato-Ku, Tokyo).

November 17–20, **Insecticides and fungicides**, Brighton (Mr W. F. P. Bishop, Conference Secretary, 87 London Road, Croydon CR0 2RF, UK)

November 17–21, **Transuranium nuclides in the environment**, San Francisco (International Atomic Agency, PO Box 590, Kartner Ring 11, A-1011, Vienna, Austria).

Reports and publications

Other countries

World Health Organization Technical Report Series. No. 564 Organization of Mental Health Services in Developing Countries—Sixteenth Report of the WHO Expert Committee on Mental Health. Pp. 41. Sw. fr. 6 No. 570 Viral Hepatitis—Report of a WHO Meeting. Pp. 51. Sw. fr. 7. Guide to the Laboratory Diagnosis of Trachoma. Prepared by the participants in a WHO Symposium. Pp. 38 Sw. fr. 12. (Geneva WHO, London HMSO, 1975.) [177]

World Health Organization Understanding Research in Nursing. By Shirley Chater. (WHO Offset Publication No. 14.) Pp. iii+36. (Geneva. World Health Organization, London: HMSO, 1975.) Sw. fr. 6. [187]

An Economic Analysis of the Co-operative Medical Services in the People's Republic of China. By Teh-wei Hu. (A publication of the John E. Fogarty International Center for Advanced Study in the Health Sciences. US Dept. of Health, Education and Welfare, Public Health Service, National Institutes of Health.) Pp. 41. (Washington, DC. Government Printing Office, 1975.) 75 cents. [187]

Canada. Department of Energy, Mines and Resources Geological Survey of Canada. Bulletin 235. Contributions to Canadian Paleontology (three papers). By A. J. Boucot, J. G. Johnson, Rolf Ludvigsen and D. G. Perry. Pp. 106 (19 plates). \$6. Bulletin 248. Conodonts of the Hull Formation, Ottawa Group (Middle Ordovician), Ontario and Quebec. By T. T. Uyeno. Pp. 31 (3 plates) \$2.50. Paper 74-21. Reconnaissance Geology of a Part of the Precambrian Shield, Northern Quebec and Northwest Territories. By F. C. Taylor. Pp. 10 \$2. Paper 74-38 Illustrations of Canadian Fossils. Cretaceous Foraminifera from Saskatchewan and Manitoba. By B. R. North and W. G. E. Caldwell. Pp. iii+35 (12 plates) \$4.20. Etude 74-42 Une Méthode d'Utilisation Quantitative de la Sonde Neutron-Neutron (Porosité) pour l'Etude des Dépôts Meubles. Par Jacques Locat. Pp. 43 \$3. Paper 74-51 Seismic Structure of the Continental Margins and Ocean Basins of Southeastern Canada. By R. Jackson, C. E. Keen and M. J. Keen. Pp. 13. Paper 74-59 Magnetic Properties of Pyrrhotite and Their Use in Applied Geology and Geophysics. By E. J. Schwarz. Pp. 24. \$3. Paper 74-62 Cavendish Township Geophysical Test Range—1973 Diamond Drilling. By D. A. Williams, W. J. Scott and A. V. Dyck. Pp. 14 \$3. Paper 75-8 Catalogue of X-ray Diffraction Patterns and Specimen Mounts on File at the Geological Survey of Canada. By M. Bonardi and R. J. Traill. Pp. 51. \$3.60 (Ottawa. Information Canada, 1974 and 1975.) [187]

Bibliography of Science Material on History of Science and Technology in Medieval India—an Introduction. By Professor A. Rahmann. Pp. 9. (New Delhi National Commission for the Compilation of History of Sciences in India, Indian National Science Academy, 1975.) [217]

Unesco. Annotated Bibliography of Textbooks and Reference Materials in Marine Sciences. Provisional edition. (Intergovernmental Oceanographic Commission Technical Series.) Pp. 109. (Paris Unesco, 1975.) [217]

Smithsonian Contributions to the Earth Sciences, No. 15 Sands in the Alboran Sea—a Model of Input in a Deep Marine Basin. By Daniel Jean Stanley, Gilbert Kelling, Juan-Antonio Vera and Harrison Sheng. Pp. iii+51 (Washington, DC Smithsonian Institution Press, 1975. For sale by US Government Printing Office) \$1.20. [227]

The Institute of Cancer Research, Philadelphia Nineteenth Scientific Report, September 1973–September 1974. Pp. 267 (Philadelphia The Institute for Cancer Research, 1975.) [237]

Unesco Intergovernmental Oceanographic Commission Technical Series, No. 13 The International Decade of Ocean Exploration (IDOE) 1971–1980. Pp. 87 (Paris Unesco, 1975.) [237]

CERN—European Organization for Nuclear Research CERN 75-8 Problems of Classical Dynamical Systems. By Thirring. Pp. 28. (Geneva CERN, 1975.) [237]

Smithsonian Year 1974 Annual Report of the Smithsonian Institution for the year ended June 30, 1974. Pp. viii+500 (Washington, DC Smithsonian Institution Press, 1974. For sale by US Government Printing Office) \$6.65 [247]

The International Federation of Institutes for Advanced Study, Stockholm—Descriptive Brochure. (Stockholm IFIAS, 1975.) [247]

Respiratory Research in the People's Republic of China. By Frederick F. Kao. (A publication of the John E. Fogarty International Center for Advanced Study in the Health Sciences US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health. Pp. xi+141 (Washington, DC: US Government Printing Office, 1975.) \$1.75 [247]

nature

September 25, 1975

Why industry and academics go their separate ways

ACADEMIC engineers say that industry

- has not the imagination to see long term potential;
- is unreliable when it comes to financial, and even moral, obligations;
- shows little interest even in projects it has backed itself;
- reacts to proposals through its accountants;
- wastes highly qualified manpower;
- is too secretive about its policies and the results of research projects.

For good measure, industry says that academics

- cannot keep to deadlines;
- get distracted from central objectives of projects;
- have too many other responsibilities to manage a project properly;
- are not keen to work to specific contract with a time scale;
- are insensitive to the realities of the market place;
- promise more than they can provide;
- have little notion of the human and environmental contexts of technology.

The caricatures described above do not come from a confidential telephone number to which malcontents could express their anonymous gripes; rather they come from a Science Research Council report published last week and entitled *Academic-Industrial Collaboration in Engineering Research* (SRC, free of charge). We hasten to add that the SRC is not urging these opinions on the community—it is simply reporting, through a panel headed by Professor E. J. Richards (Loughborough University), the common stereotyped views that still circulate widely and undoubtedly have some substance.

The panel, like many predecessors, is concerned at the “far from satisfactory” links between university and industry, and particularly links which develop, or fail to develop, when universities look to the SRC for support in engineering research ventures which are too speculative and risky for industry to pursue in its own laboratories. The problem is put more simply and chauvinistically by politicians and newspapers—“Why do we have so many good ideas and then waste them or let a foreign company exploit them?”

This popular viewpoint may be quite wrong. British companies may steer clear of certain ideas with justification. The panel acknowledges that in some industries (computers, for instance) the collaboration is much easier to bring about. And in some cases the international nature of corporations makes simple-minded talk about British universities benefitting British industry dated and meaningless. But there is no denying that there is still much uneasiness that universities and industry go their separate, mutually incomprehending ways far too much.

The panel sees three serious gaps in the present framework for research support:

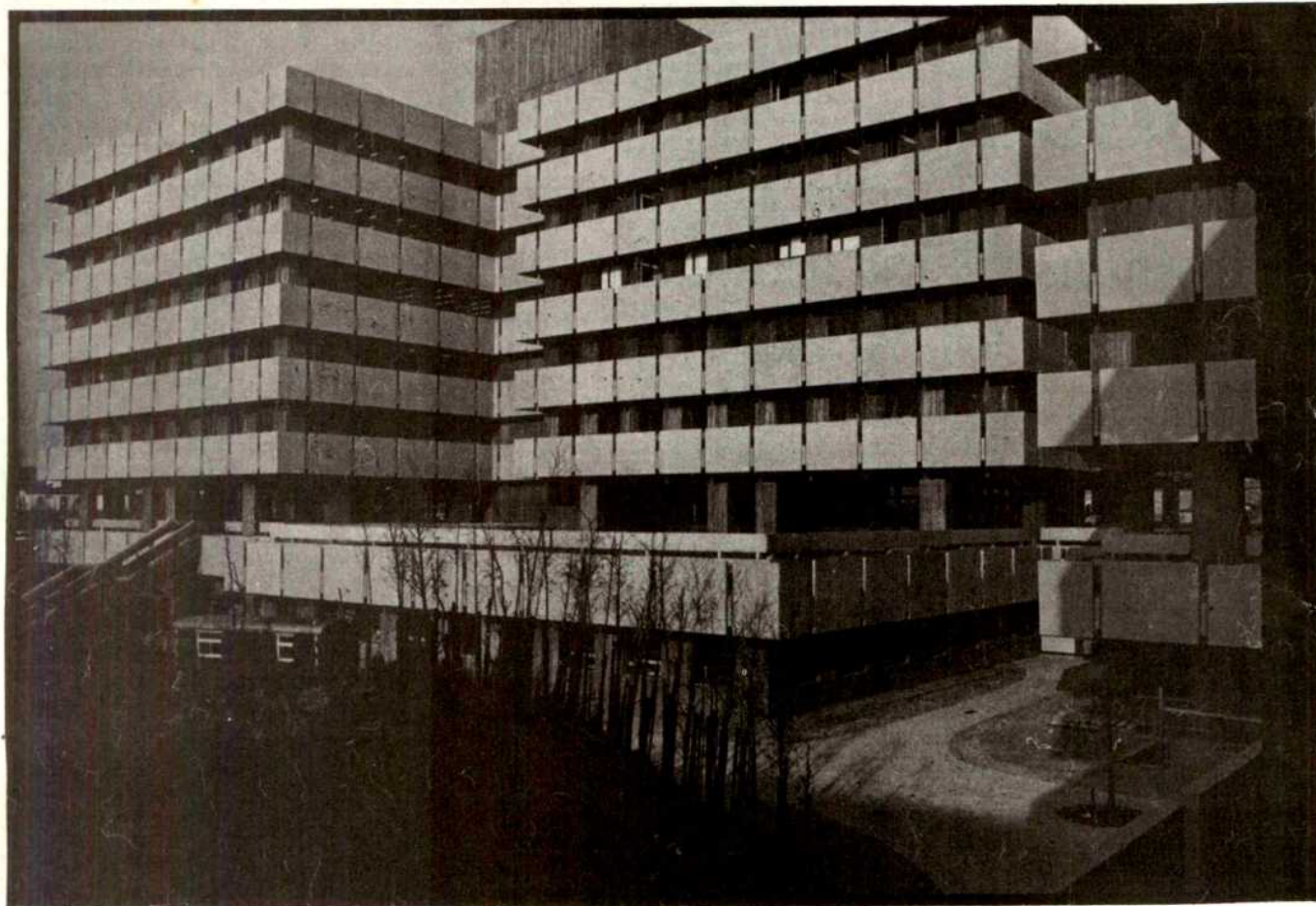
- A pre-development gap between researcher and industrialist concerning the time at which a research project can be seen to have an outcome. The academic scents success for an idea long before an industrialist is convinced; the panel urges that bridging work be given much greater support, by either the SRC, the universities, industry or research associations. This is the central message for SRC who have traditionally only supported research.
- An evaluation gap between researchers and industrialists in the following-through of research results. The panel points an accusing finger at the SRC whose record is “very poor” in the dissemination of information so that industry can get early warning of developments.
- An identification gap; it seems that academics would positively welcome a more interventionist approach by the SRC in policy formation and implementation. There is much, says the panel, that the Engineering Board (of the SRC) could do to improve its own ability to identify research needs.

It is striking how much of what the panel says boils down to one problem—adequate communications. Perhaps the most telling section of the report runs: “... success [in collaboration] depends strongly on the compatibility of the individuals in contact, and their enthusiasm for the project. Clearly defined objectives and responsibilities are also important, but rank second to satisfactory human relationships”.

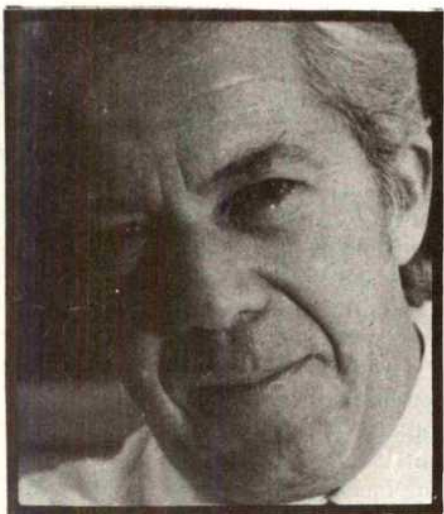
The panel was in no position to do more than make recommendations relevant to the SRC’s policy, but it seems abundantly clear that at some time two broader issues will have to be faced.

First, are not engineering departments in many universities too isolated? The separation of university entrants into mathematicians, scientists, and engineers, each pursuing separate educational paths (and sometimes attending almost identical lectures) not only makes it more difficult for the student to sample widely and choose appropriately but also encourages an artificial erection of barriers around engineering which is bound to lower the general ability to communicate.

Second, the amount of movement in mid-career between university and industry, either for a year or for an indefinite period, is still pitifully small. It is certainly possible to point to short term exchanges, but if communication is to be improved it must be on the basis of understanding the constraints under which the ‘other side’ works. This can hardly be done in a few weeks, but rather requires people to stay long enough not just to be involved in the making of decisions, but also to have to live with the consequences of those decisions. □



The International Institute of Cellular and Molecular Pathology (above) was inaugurated as a centre for medical research last April. Christian de Duve (below) is president of its governing body as well as chairman of the executive committee and in those capacities he directs the activities of the ICMP. The institute is already acting as a honey-pot for expatriate Belgian scientists but it is de Duve's problem to try and make the institute live up to its 'International' intent. Peter Newmark reports from Brussels.



THE ICMP will probably be international before it is truly national, having been born out of the long standing Belgian failure to integrate the French and Flemish-speaking communities. Separatist movements at the Université Catholique de Louvain (UCL) triggered off such disruptions in 1968 that co-existence on one campus became impossible. It was during the ensuing upheaval that the idea of the ICMP arose, along with a government plan to found a separate French-speaking UCL on Walloon territory. The outcome of subsequent deliberations was a brand new campus in a Brussels suburb. On this campus are sited a 900-bed hospital, the new UCL medical school and the ICMP.

In spite of the very close links between the ICMP and the medical school, including the fact that all the senior members of the former hold chairs at the UCL, the institute aspires to autonomy, and is well on the way to it. Thus it is registered as an independent body, has its own charter and governing body and maintains a Scientific Advisory Board of international notables who are consulted before any important appointments are made. (Advice in the negative from them has already been followed on occasion.) In addition the official language of the ICMP is English.

All research at the ICMP is intended to be medically orientated but with

some categorisation into basic and applied projects. The organisation is described, perhaps more for the benefit of laymen and fund-raising than for experienced scientists, as consisting of a central core of basic scientists on to which are attached groups that are tackling more applied problems—the promised advantage being that many diverse groups can obtain adequate basic support from the multiple skills of the core.

So far the majority of the staff, at present numbering about 150 including supporting personnel, belong to the 'central core'. They divide into four main groups. Largest by far is de Duve's biochemistry group which concentrates its work on subcellular particles and the metabolism of both glycogen (under H. G. Hers) and connective tissue (under G. Vaes). The experimental medicine group is the next largest. Led by J. F. Heremans, it provides the immunological know-how of the ICMP and has an active interest in various proteins of body fluids. Third, there is a general pathology group operating in the field of endocrinology, particularly thyroid hormones, under M. de Visscher. And finally there is C. Cocito's small microbiology and molecular genetics group. Each of these four groups was located separately until the ICMP united them and each is very enthusiastic about its new brotherhood.

But what of the new groups that de Duve is hoping to graft in to, or preferably on to, the existing 'central core'? So far a large grant from a French drug company has enabled A. Trouet to set up an experimental chemotherapy unit, T. Boon is about to join the institute to start up a cellular genetics group that will concentrate on teratocarcinomas and there are advanced plans for a parasitology group backed by the WHO.

Noticeable at this stage largely for their absence are any new clinically orientated research groups. Since these are part not only of the design but also of the justification of the ICP, they will have to be started in due course. The main question that concerns de Duve at present is whether he can find suitable staff to originate and run new groups.

In his favour are the fine facilities of the ICP, the permanent posts that can be offered and the fact that de Duve (no band-waggoner himself) is prepared to back almost any line of medical research provided he finds the right person. Against him is the considerable problem of attracting the foreign candidates that de Duve is after.

Why look for foreigners? In the first place there simply are not enough top-class Belgian scientists: second, there is the 'International' label to live up to. But over and above those reasons is de Duve's profound belief in the value of mobility among scientists. This belief is nurtured by de Duve's long association with the Rockefeller Institute where he spends about half his time and will continue to do so. In fact, the Rockefeller clearly plays a godparent role to the ICP, acting as a general model, having been a temporary home for most of the senior staff at one time or another and providing two of the members of the Scientific Advisory Board.

Also in line with de Duve's encouragement of mobility will be his policy of only giving tenured posts to the most senior of newly appointed staff. Others will be taken on limited appointments of a few years, some on a fellowship programme that is about to start.

It remains to be seen how successful this policy will be at a time when even the best young scientists are not prepared to take untenured posts except as a last resort.

Professor de Duve is a modest man, courageous enough to try and build up an international institute in a city which, apart from becoming the Washington of the European Economic Community, has few outstanding attractions. His Nobel Prize last year has not only brought him fame and fortune but has been an exceptionally timely boost for the ICP. □

THOMAS JUKES



Evolutionists brought to book

THIS year is the fiftieth anniversary of the "Scopes Trial" in Dayton, Tennessee, when the issue of teaching evolution was argued between William Jennings Bryan and Clarence Darrow. The trial was showbiz, not, as some have thought, a public martyrdom of Scopes. It was put "on the air" in one of the first coast-to-coast network radio broadcasts. Scopes was fined \$100, this was paid by the *Baltimore Sun*, and Scopes left Tennessee to accept a graduate fellowship (a rarity in those days!) offered because of his new fame. The evolutionists got their story to the public. At the time, it seemed that Bryan succeeded in convicting the defendant but that Darrow triumphed ideologically. Half a century later, the story of the trial, as told by H. L. Mencken, sounds archaic to the young, and nostalgic to their parents.

But there is an uneasy feeling that, as far as school textbooks in the USA are concerned, evolutionists may have won the battle of Dayton, but not the war. For publishers live by selling books, and it is poor for business when parents object to a science textbook: the competition would gladly omit "controversial material" to win the contract. As an "evaluator" for the California State Textbook Commission, I was prepared to resist the opponents of evolution on the State Board of Education. I found, however, that there was very little mention of evolution in the books. Publishers will not prepare a special "California edition", so the result is that the national versions have little to say about the subject, with the notable exception which Professor G. G. Simpson has noted, of those published by Harcourt Brace Jovanovich. □

The "textbooks" published by the Creation Science Research Center of San Diego have plenty to say, however. Much of their content is artfully directed towards attempts to discredit geological findings and isotopic dating. Few physicists, palaeontologists and geologists wish to get into arguments emanating from those who truly believe in a special creation that took place instantaneously 6,000 years ago. But I think we may learn a simplified approach from the following famous exchange in the Scopes trial:

DARROW: Mr Bryan, do you believe that the first woman was Eve?

BRYAN: Yes.

DARROW: Do you believe that she was literally made out of Adam's rib?

BRYAN: I do.

DARROW: Did you ever discover where Cain got his wife?

BRYAN: No sir; I leave the agnostics to hunt for her.

Fifty years later, creationists are passing the hat for the latest of their expeditions to look for the remains of Noah's Ark on Mount Ararat. Surely if they wish to argue about geology, we are entitled to ask a few questions about the Ark, its dimensions, and the time schedule of the Flood. These are set forth with great precision in the Book of Genesis, in an account which also contains one of the most cherished of allegories—that of the dove of peace.

Our creationist friends, literal and unimaginative, are more concerned with looking for pieces of wood. Very well. Let us ask them a few questions about ecology in its most compact form—the living quarters on the Ark for all terrestrial species (including koala bears, *Plasmodia*, tapeworms and tigers), plus their food supply and maintenance for 400 days, in a volume of 44 000 m³. There is also the minor question of what happened to about 1.6 × 10⁹ km³ of water when the flood receded. This much water would be needed to cover the Earth to a depth of 3,000 m, an increase in water level that would be actually only about half that required to submerge and drown "every living substance . . . which was upon the face of the ground."

But I digress. In some religious sects, there is still a zealous opposition to teaching evolution, and the effects of this have been recently visible in the states of California, Texas and Washington. The subject of evolution is one of our great cultural heritages, which has now expanded to encompass biology in a single network of molecules, and the recent problems in the USA are a lesson to all of us to keep up-to-date on knowledge of the fossil record and the methods of isotopic dating of rocks. □

ASTRONOMY, cradled in superstition, and fed in infancy by the fears of kings, only became a science when it dissociated itself from astrology and restricted itself to matters capable of mechanistic interpretation. Other sciences, including most fields of medicine, have likewise weaned themselves from their infant diets of credulity and crude utility. In consequence, chemistry, mathematics and physics have become reputable disciplines whose general scope and aim could not be misrepresented in public documents without exciting wide comment.

Why is the genetics of man not accorded the standards of public scrutiny and stock-taking normally afforded to radioastronomy, high-energy physics, and the genetics of flies? It can hardly be mere difficulty, for the basic mechanisms of genetics are, in principle, far simpler than those invoked for stars and atoms, and the genetics of man is only marginally more remarkable than that of the fly. Until we can diagnose the cause of this contagious mediocrity we cannot expect any cure, and without some attempt at cure we cannot expect that workers of the calibre expected in those who listen to the skies, or look for portents in bubble chambers, will be attracted to this field. The reputed mediocrity of physicians, a belief widespread among those who are not physicians, is hardly a reason, for immunology, virology, and numerous other specialised fields in and around medicine are progressing well.

The reason must be in ourselves. While we can accept, after many generations, the peripheral isolation of the Earth, and even the Sun, the full implications of evolution and of natural variability are too much. Genetic disorders are rare, and only a minority are amenable to elimination, to prevention, or to treatment. They have been with us throughout our evolution, and most are likely to stay with us for as long. They require the same basic facilities for research, for diagnosis, for treatment, and even for elimination, as do other forms of disease.

Genetic disorders are, by definition, those to which the flesh is heir, and cover all disease. In practice it is convenient to use the word 'genetic' to cover those disorders consequent upon some hereditary unit, or pair of units, which is usually either sufficient, or necessary, for the manifestation of disease, as in the genic and chromosomal disorders. Victims include those who, by chance, were conceived without the alleles necessary for synthesising pigment in the eye and skin, or, by mischance, were conceived with the handicap of an extra chromosome.

Even here words can be confusing,

for albinos rarely have inherited any noticeable disorder from their ancestors, and those burdened by an extra chromosome are usually born to parents with a regular allocation. But the term genetic, implying an inborn qualitative or quantitative abnormality in their nuclear apparatus which is sufficient to cause distress, is useful and rarely ambiguous. Obviously, the environment will influence affliction, but, within any regular environment, any inability to cope with minor injury, sunlight, or conventional food can be a sufficient handicap to qualify for the term 'disease'.

Genetic facts?

Once the word genetic is extended to cover any inborn predisposition, whether to illness, to aptitude, or to crime, difficulties arise which can only be removed by a less casual use of words. A recent publication from the US National Institute of General Medical Sciences, National Institutes of Health, (*What are the facts about genetic disease?*, with the strange subtitle, *Most ubiquitous of all human maladies*) does nothing to lighten the genetic load of confusion, and its authors have done a serious disservice to those—many of whom are featured in photographs in this booklet—who are striving for an orderly application of genetic knowledge to man.

The book tell us (p. 6) that 15 million Americans have birth defects, and that 80% of these "carry true genetic diseases due wholly or partly to defective genes or chromosomes". The genetic load is then elaborated to cover 100,000 abortions and 40% of infant deaths; each of us (presumably US citizens) carries "between five and eight recessive genes for serious genetic effects" and each married couple has a 3% risk of having a genetically defective child. The opposite page is a little less exact with recessives, giving the range as "between one and ten", and shows some normal looking individuals and a superimposed pedigree of the extremely rare and variable Hartnup disease, stating that the normal siblings are at risk of transmitting it to their offspring—a risk too low to justify anxiety, or even the use of the word risk.

Next, disorders of infancy are shown to cause more lost years of life than disorders of the aged. Single gene defects are said to occur in 1.8 to 2% of all births. But the "overall incidence of recognised genetic diseases is 4.8 to 5.0% of all live births", a curiously exact estimate, but one which seems out by a factor of 10. We are told that

"authorities now estimate that seven to eight per cent of the US population is adversely affected by one or another form of heritable disease" (p 14) while the next page includes gout and diabetes as genetic diseases, which certainly helps the percentages. We are probably all potential diabetics. Heart disease emerges again on page 21: this time 20% of cases are attributable to one of three genes and 5% are polygenic (what about the other 75%; can anything be non-genetic and non-polygenic?). Those three unspecified genes are said to be the most common disease-producing genes. Estimates of hard cash are made with equal abandon. Death from Tay-Sachs disease is costed at \$35,000, which seems a lot unless it includes the artificial piping of both food and air to the dying. Earlier, the booklet states that "specific genetic factors are involved in approximately one-fifth of all heart attacks". What does this mean? Why not incriminate the Y chromosome, which is surely genetic, and is found in at least four fifths of victims of heart attack?

How can the economic custodians of medical research in a country which has such numerous claims to excellence in both aim and achievement, be associated with the publication of such a confused and contradictory document? For whom can it be meant? Why are the achievements made by other routes, such as safe blood transfusion, the 'cure' of rhesus disease by exchange transfusion, and its prevention by immunisation, not worthy of mention? Are not the triumphs of haemophilic therapy worth a sentence? The US decision to funnel funds into 10 centres seems strange, since excellence cannot be constrained, even in smaller countries; is this booklet for congressmen who think their constituents neglected? Or is it what the *Observer* calls "research by public hysteria"?

Genetic research needs money, and the excellence of this research in the US would seem to have suffered even more than that in most other countries during recent years. A pre-occupation with costing, with short term aims, and the throwing around of wild estimates of such measures of the genetic load as the number of recessives per gamete, or the number of dollars per death, does little to reassure admirers of American genetics that the future will be worthy of the past.

It is tempting to dismiss this as a mere manifestation of bureaucratic immaturity, hardly worthy of comment. The matter is however, too important to be glossed over in this way. There is nothing more important than the orderly survival and civilisation of our own species.

J. H. Edwards

international news

THE absence of an official statement from the World Meteorological Organisation (WMO) after the recent meeting of its working group on stratospheric and mesospheric problems is being interpreted as an indication that WMO sees no danger of imminent disaster caused by the depletion of the Earth's protective ozone layer. This is in spite of calculations presented to the group to the effect that long term depletion of stratospheric ozone by the continued release of freons at 1972 levels could amount to more than 10%, and that ultraviolet radiation reaching the ground could increase by 2% for every 1% decrease in ozone. The group apparently wrote into its deliberations that it was working on a "factor of uncertainty" of about 2, which, at worst, would indicate a 20% depletion of stratospheric ozone, with a corresponding increase of 40% in radiation levels. This would be serious by any estimate.

The working group on stratospheric and mesospheric problems was set up under WMO's Commission for Atmospheric Sciences, and included many of the world's leading experts in this field: physicists, stratosphere chemists, experts on ozone, on the physical and chemical behaviour of the stratosphere,

WMO silent on ozone depletion

from Peter Collins, Geneva

and those concerned with evolving models for the study of what goes on in the stratosphere. The group was concentrating principally on the chlorofluoromethanes (freons) resulting from the use of certain aerosols and certain types of refrigerator, and which have recently been the subject of alarm, especially in the US. It is the first time that these substances have been considered by WMO at this level and it is indicative of the increasing attention being paid there to the broader environmental aspects of air pollution.

As summed up by a participant, the meeting concluded that calculations on an average, world-wide basis, ignoring latitude and the longitude and using one-dimensional models, indicate that the present anthropogenic contribution to the depletion of ozone by freon 11 (CFCl_3) and freon 12 (CF_2Cl_2) already in the stratosphere is 0.5 to 1.00% of the total amount of ozone. The long-term steady state effects of continued release of these substances at 1972

world rates would result in about a 10% depletion with an average factor of uncertainty of about 2. The effects of this 10% on the thermal regime of the stratosphere might be noticeable but are still not clear; the consequences of any such changes on tropospheric weather systems are likewise uncertain. It has also been estimated that a 1% decrease in the total ozone amount would permit a 2% increase in ultraviolet radiation at ground level, always assuming clear weather.

The group also discussed other pollutants such as bromine and nitric oxides, but it has been pointed out that the freons arising from human activities at the Earth's surface are very much more serious than anything that could happen from the use of aircraft, including nitric oxide emissions which are of such concern to the anti Concorde lobby. No official report of the meeting is likely to be forthcoming for some months, and even then it is likely to express the conclusions with great caution. One reason for this is that we still know very little about what goes on, chemically and physically, in the stratosphere. Here, the group did express itself clearly in agreeing on the need for more data from observation. □

NOT quite two years after the publication of its third and final report recommending major changes in the formulation and implementation of science policy in Canada, the Senate's special committee on science policy is preparing for an investigation of how effectively those changes have been carried out.

Senator Maurice Lamontagne, the committee's chairman, says letters went out in September to the Ministry of State for Science and Technology (which was created in part as a result of the committee's recommendation) and the Science Council of Canada, asking them to prepare briefs for a new set of hearings to begin in November.

Since the publication of the last volume of its report in the autumn of 1973, the committee has been concerned with planning a major conference on future research for decision makers at various levels of national life to attend. That involved preparing background papers for an area of interest largely neglected by other institutions in Canada. The committee also proposed establishment of two

institutions: a Canadian Centre for Future Studies and a commission called Futures Canada.

Much to its obvious chagrin, the federal government decided last summer to accept the thrust of the Senate committee's recommendations, but to

Lamontagne revisited

from David Spurgeon, Ottawa

take the matter out of its hands and entrust it to the recently formed Institute for Research on Public Policy.

This left the committee without a job.

Senator Lamontagne's response was to propose a return to its former mandate, "not to start a new inquiry but to uncover the areas where our demand for change and improvement has been met with minimum compliance, or simply with autocratic and bureaucratic rejection."

Even before the new hearings begin,

the Senator is less than pleased about governmental response to his recommendations. He sees MOSST (the science ministry) as possessed of "suicidal" tendencies (some 30 senior employees were let go recently), and he finds no technological strategy developed by the Department of Industry. "Government support for science, technology and innovation," he told the Senate, "continues to be inadequate, uncoordinated and wasteful."

The Science Council of Canada may welcome the committee's new activity, because it seems to have been eclipsed more and more as a result of the creation of MOSST. But it is difficult to believe either the government or MOSST will be exactly overjoyed. The ministry seems to have been slipping slowly into oblivion in recent months, and just as the new part-time minister, C. M. Drury (who also held the portfolio of public works) had begun to counteract this drift with a public definition of its role, he found himself appointed interim finance minister as a result of the resignation of the former minister, John Turner.

Pauling comes in from the cold

PRESIDENT Ford last week invited 13 scientists to the White House to receive the National Medal of Science, the most prestigious scientific prize offered by the federal government. For one of the recipients, Linus Pauling, the event had added significance, for it marked the end of a long and bitter period during which Pauling had been virtually ostracised by the White House for his outspoken political opinions and his opposition to nuclear weapons.

Although he has received almost every possible scientific accolade, including two Nobel Prizes, Pauling has suffered a number of direct reprisals for his political views, and he has repeatedly been ignored when the National Medals of Science have been handed out. The latest such rebuffs came during the Nixon Administration when Nixon, who was not known for honouring his enemies, twice overruled Pauling's nomination for the prize.

Pauling's political troubles began in the early 1950s, during the anti-communist witch hunts which culminated in the rampagings of Senator Joe McCarthy. Accused of being a Communist sympathiser, he was denied a passport in 1952 to travel to Britain for a meeting of the Royal Society, and he was again refused a passport a year later. He was also refused a government research grant in the early 1950s for political reasons.

Such overt reprisals lessened a little after he was awarded the Nobel Prize for chemistry in 1954, in recognition of his pioneering work in unravelling the nature of the chemical bond. He was, at least, granted a passport. But Pauling continued to needle successive Administrations by attacking nuclear weapons testing, foreign policy and, in the 1960s, the Vietnam war. Although his actions brought rebuffs at home, Pauling's political activities brought him the Nobel Peace Prize in 1962 and the International Lenin Peace Prize in 1971.

There was, however, one occasion on which the White House temporarily suspended hostilities against Pauling. Soon after he was elected, President Kennedy invited Pauling, along with every other American Nobel Prize-winner, to a White House dinner. Pauling accepted, but before attending the event, he led a protest demonstration outside the White House gates against nuclear weapons testing.

The citation which accompanied his National Medal of Science states that the award is "For the extraordinary scope and power of his imagination, which has led to basic contributions in such diverse fields as structural chem-



Pauling at the White House with a friend (pic: Washington Post).

istry and the nature of chemical bonding, molecular biology, immunology, and the nature of genetic diseases". Pauling, who is now 74, accepted the prize with a broad grin, accompanied by the clicking of a multitude of cameras.

Other recipients of the award were Britton Chance (University of Pennsylvania), Erwin Chargaff (Columbia University), James Neel (University of Michigan), James Shannon (former Director of the National Institutes of Health), Rudolf Kompfer (Stanford University), Ralph Peck (University of Illinois), Abel Wolman (Johns Hopkins University, now retired), Kurt Godel (Institute for Advanced Study), Nicolaas Bloembergen (Harvard University), Paul Flory (Stanford University), William Fowler (California Institute of Technology) and Kenneth Pitzer (University of California, Berkeley).

● At the annual International Astronautics Federation congress this week in Lisbon, the Guggenheim International Astronautics Prize, awarded by the International Astronautics Academy, goes to Oleg G. Gazenko, now Director of the Institute of Biomedical Problems in Moscow. More than anyone else in either the US or Soviet space programmes, he has made a concerted attack on the daunting problems of placing and maintaining man in space—and it is now hard to remember that before 1957 many

physiologists were convinced that such a venture would only prove a costly catastrophe. The line of development conceived and carried through by Gazenko and his colleagues can be considered to have culminated in the effective compromise of the Apollo-Soyuz joint test project, in which the Soviet life support philosophy and that of NASA were blended rather than unilaterally bent.

Oleg Gazenko's involvement with the physiological problems of space flight and life support for long manned spaceflights goes back beyond 1957, to the time when the first Soviet physiological experiment was carried out in space—involving the dog Laika in the second *Sputnik*. The choice of the dog as the test animal for pioneering *in vivo* experiments in space may be attributed to Gazenko, although it conformed equally with classical Pavlovian behaviourism favoured by the Soviet medical establishment.

Since the mid-1960s Gazenko has been in the thick of the project (finally agreed in principle in 1964) for joint publication of US and Soviet space medical and biological experience (*Foundations of Space Biology and Medicine*). Recently, American research and competence in space biology and medicine has more than caught up and it has assumed for a year or two that Gazenko had lost status. This award should set the record straight. □

AN extraordinary period of expansion in the life of Israeli universities is coming to an end. After a quarter of a century during which student enrolment has increased almost 40 fold (in contrast to a five-fold increase in the general population), it is now up by only 5 or 6% a year. And severe financial problems have virtually brought an end to the spate of multi-million pound building projects so characteristic of the last 25 years.

Not all universities have been affected in the same way by the new situation. Though the 50 year old Hebrew University of Jerusalem is actually expecting a decline in enrolment this year, the newer institutions of higher learning in Haifa, Tel Aviv and Beersheba are still growing, albeit at a slower pace than previously.

Off the record, some professors argue that Israel does not need even its existing seven institutions of higher learning to handle the current student population of some 60,000 (which gives Israel about the same percentage of university students as the countries of western Europe). Since, however, it would be politically impossible to shut one down, all the Council for Higher Education could do was to ban the establishment of new universities (in the foreseeable future) and to crack down on plans to create new faculties at existing universities, particularly where they duplicate programmes already available elsewhere in Israel.

Reaction to the situation has varied from campus to campus. The President-elect of the Weizmann Institute, Professor Michael Sela thinks that Israeli institutions of higher learning should, in the circumstances, "use their shrinking resources to raise academic standards rather than add floor space". And Professor M. Z. Kaddari, until recently Rector of Bar-Ilan University, wants to cut down on the intake of students, not only because of budgetary problems but also "to avoid the creation of a disillusioned academic proletariat".

Naturally enough, the President of the Technion Institute of Technology, Amos Horev, believes that, regardless of the number of students, a greater percentage of them should be studying engineering lest a shortage of engineers frustrate Government plans to double Israeli industrial output by 1980.

Whatever the logic of Horev's argument, priorities in the sphere of higher education will have to be more clearly defined than they have been in the past, when money seemed to be available for everything at once. The boom is over.

● Despite their financial problems, or possibly because of them, institutions of higher learning are strengthening their ties with local industry. A recent con-

sequence of such cooperation is the Microviscosimeter MV-1, an Israeli instrument expected to facilitate the early detection of leukaemia, which was this week named one of the 100 most innovative products of the year by *Industrial Research*, a US magazine.

The instrument was developed by the Elscint Company of Haifa on the basis of studies carried out by young researchers Michael Inbar and Meir Shnitzky of the Weizmann Institute's Laboratory of Membranes and Bioregulation. It grew out of an

Letter from Israel

from Nechemia Meyers

apparent correlation discovered by Dr Inbar and Dr Shnitzky between the level of cholesterol in the cell surface membrane and the blood, and the development of leukaemia.

Named Israel's Outstanding Exporter of 1974 for its large overseas sales of medical and laboratory equipment, Elscint is particularly proud of the fact that its MV-L is sharing honours this week with instruments created by a group of international giants.

● Another achievement of local, science-based industry unveiled recently is a mobile pilot plant for processing sewage that provides as end products both purified water (suitable for irrigation and almost suitable for human consumption) and concentrated algae for use as high-protein animal feed.

Built by the Membrane Division of Israel Desalination Engineering Ltd (IDE), under the supervision of Dr S. Sachs, the installation, unlike treatment plants now in operation, does not require the introduction of algae-killing lime or alum into its oxidation pools. Instead, it concentrates and purifies the sewage by low pressure ultrafiltration, using membranes developed specifically for the task.

It is not yet possible to estimate the cost of building and operating IDE units on a commercial basis, but an important factor in costs—the energy input required—is relatively low because the purification process can be carried out at a pressure of 7 or 8 atmospheres, as compared to 60 or 70 atmospheres in conventional reverse-osmosis systems. Moreover, the low pressure permits the unit to be built with less expensive materials than would otherwise be required.

When expenses are calculated, one must also consider the fact that the algae 'left over' should bring in substantial sums of money.

Economic considerations in Israel

may be rather different from those of other countries, as additional water supplies are urgently required here, almost regardless of cost. But both the Treasury and the taxpayers would be most grateful if recycling a larger part of Israel's sewage made it possible to delay the construction of large scale and very expensive desalination plants.

● A more controversial scheme for increasing water supplies has come from the Ministry of Agriculture's Soil Conservation and Drainage Department. Ministry experts claim that transforming 85,000 acres of shrub-covered Galilee hillsides into pasture land would save 85 million cubic metres of water because grass requires less moisture than shrubs and the "surplus rain" would seep down into the aquifer. Moreover, they add, the new grasslands would provide enough forage for raising 24,500 cattle each year, as well as creating an excellent recreation area.

The scheme has, however, been severely criticised by, among others, Professor Zeev Raveh of the Technion Institute Faculty of Agricultural Engineering. "If the plan were implemented," he declares, "it would supply the country with very little additional water or meat, and, far from creating a new recreational area, it would gravely endanger the ecological balance of one of Israel's few remaining focuses of natural beauty and rich biological diversity."

Pointing out that the Ministry of Agriculture plan has so far only been tested on a carefully tended, one and a quarter acre plot, Professor Naveh doubts very much whether the lessons learned there are applicable over a much wider and more diverse area. He notes, for example, that uprooting shrubs is a very expensive process if it is to be effective (costing several times more than was estimated by Ministry planners) and, he adds, there is no certainty that the new vegetation that takes over will be suitable for grazing purposes.

Though a few months ago it was planned to implement the Ministry of Agriculture scheme at the rate of 8,500 acres a year, public protests have prompted the Government to undertake further studies before uprooting any shrubs on a large scale. At the same time, measures will undoubtedly be taken to foster the multipurpose use of Israel's extremely limited areas of land. Professor Naveh, for his part, thinks that they must be carefully designed to suit the requirements and possibilities of each site so as to achieve a maximum degree of economic benefit (in the form of increased water yields, grazing land and timber) while ensuring that key elements in the ecosystem are not undermined.

correspondence

Moon

SIR,—One must assume that at the beginning of each year the Editor of *Nature* writes in his diary, in the appropriate place, "Be sure to include an article designed to discourage readers from attending the International Conference on the Unity of the Sciences". Having attended last year's ICUS conference, I can vouch for the fact that the majority of the eminent names mentioned in the advance publicity did in fact attend the conference and make contributions. As far as I am aware, no-one present was dissatisfied at the proceedings, and at the end I was left with the impression that the conference had succeeded in achieving the aim of any conference, namely of allowing free exchange of ideas on important topics.

Regarding the sponsorship of the conference, from his opening address I gained the impression that at any rate some of the Rev. Moon's motives are at least as worthy as those of such well known conference sponsors as the armed forces. As to the rest, news (as reported by you) is capable of being distorted in many ways, but whatever the true situation may be, I find it difficult to accept from a reputable scientific journal something which one cannot interpret as anything other than a deliberate attempt, for ideological reasons, to sabotage a conference by discouraging potential participants from attending.

Yours faithfully,

B. D. JOSEPHSON

Cambridge, UK

Silly in the head

SIR,—Regarding the "silly season for newspapers" as discussed in *Nature* (August 28), how about the headline for a recent Independent Television News at Ten—"British scientists discover origin of Universe"?

Yours faithfully,

JOHN WOODSIDE

Cambridge, UK

SIR,—Herewith some suggestions for your silly headlines list:

Britain fastest in entropy acceleration studies—official
Plutonium for Arabs in genetic manipulation research deal
Pugwash scientists named in SALT talks sell-out allegations
Ice-age predictions snowball as funds frozen

Nature readers in shock titles scandal
—alleges Longford

Yours faithfully,

BERNARD MEARES

Copenhagen, Denmark

Stop metrication

SIR,—On August 28 you referred to the pointless advance of metrication. There is one area where good sense has a foothold, even in France. Whether in Notre Dame or the humblest village church, organ pipes are still measured in feet. I could not think of foundation stops of 2.4 m or adding the brilliance of a 0.3 m rank.

Common sense still prevails over logic in one area—I am sure that there must be others.

D. C. HARDWICK

London, UK



CAB directors

SIR,—It is unfortunate that your correspondent in his article on the Commonwealth Agricultural Bureaux (August 14) spoiled an otherwise factual and informative piece by an inclusion of quite unmerited criticism of CAB directors.

As your correspondent says "the bureaux have maintained and justified their reputation for providing the world's best and most comprehensive source of information in the fields they cover". The reputation has been gained not by the central management but by the individual bureaux under the management of the directors. That changes are needed to modernise the bureaux structure is acknowledged by the staff, and the Institution of Professional Civil Servants (IPCS) has put forward on their behalf proposals which we hope the CAB management will adopt but which will be rather

different from those proposed by the Civil Service Department review team.

The CSD review team made recommendations which implied criticism of the central management rather than of the staff or the directors of bureaux. The misuse of computer printing, resulting in an increase in the time taken to publish abstracts as compared with the time when directors were completely responsible for publication, is an example. Clearly there is room for improvement in the management of computer services. Certainly to the extent that it is possible, collaboration with other agencies doing similar work is desirable. But your correspondent is wrong in assuming that there is not already collaboration with AGRIS, the bureaux already scan UK journals for AGRIS and make no charge for this service.

The report of the review team stress on the economies in staffing which would result from operating the abstracting and editorial services on a commercial basis. We believe that this could be achieved only by lowering the standards of the service and this would seriously reduce its value to its users. An important feature of the CAB service is its abstract enrichment and this could not be maintained if commercial viability were the first consideration. The review team made no attempt to test its assumptions by market survey. Enquiries we have made from a necessarily small sample of eminent scientists in the agricultural field have all met with the response that the CAB abstracts must be maintained at their present high quality if they are to be of value. By all means let us have additional effort to market the CAB's products (and the staff will not be greatly concerned whether this job is described as "marketing" or "circulation and services") but the article for sale must be first class.

Whatever changes are made in procedures and staff structures they will be successful only if they have the whole-hearted cooperation of the staff. A lowering of standards would lead to loss of job satisfaction for the staff and a drop in morale. Staff morale will not be improved by suggestions such as those made by your correspondent that the staff are responsible for the difficulties which are solely attributable to bad management.

EDWARD HEWLETT

IPCS, London, UK

news and views

LESS than a year after their discovery it was widely accepted that pulsars are rotating neutron stars, with high magnetic fields and consequently with a very energetic co-rotating magnetosphere. Progress beyond this basic interpretation has been slow, not through a lack of experimental data but through the sheer unfamiliarity of the physics involved in these strange objects. The solid state physics has to deal with densities approaching $10^{14} \text{ g cm}^{-3}$, with a composition varying from a neutron superfluid to a crystalline crust mainly composed of iron nuclei. The very strong magnetic field is sustained by the superconductivity of the neutron star, and itself modifies the crystalline structure of the crust. Conditions in the magnetosphere are even more bizarre, with complete charge separation and vacuum spark gaps as possible features.

The difficulty in interpreting the radio pulses, which provide our only observational material on nearly all of the pulsars, is that they originate in only a local region of the magnetosphere, and their radiation processes are limited indicators of the behaviour of the rest of the neutron star. Furthermore, they have very complex structures, which we cannot easily interpret without knowing more about the radiation process and the precise location of the emitter in the magnetosphere.

Recent work on pulsars at Jodrell Bank has used the timing of the radio pulses to investigate the slow-down of rotation of the neutron star. The

Physics of pulsars

from F. G. Smith

slow-down is a consequence of radiation from the rotating dipole magnetic field, so that this investigation was using the radio pulses as an indicator of conditions within the star. Surprisingly it led to the suggestion that the magnetic field decays with a time constant of a few million years, which is in conflict with present theories of the superconducting interior of neutron stars.

Another investigation, also related to the age and evolution of pulsars, but which refers to the magnetosphere rather than the star itself, is reported in this issue of *Nature*. Ritchings and Lyne (page 293) have found a new orderliness in the apparent disorder of the structure within radio pulses. Some pulsars, and particularly the older ones as shown by the previous investigation, have "drifting sub-pulses". An individual radio pulse in these pulsars consists of one or two narrow sub-pulses, which can be seen in several consecutive pulses but at slowly varying times within the pulses. The "drift" in time can be either earlier or later, although until recently it was thought that all drifts were earlier. The remarkable observation now reported is that these two cat-

egories are simply divided between those pulsars with slowly lengthening periods, which drift earlier, and those with rapidly lengthening periods, which drift later.

The most important conclusion must be that there is after all some simple classification and evolution in the pulsars, whose behaviour may perhaps be regulated mainly by the strength of their magnetic fields. We do not know at all how the drifting itself occurs, although there have been some interesting suggestions about the drifting of discharge paths across a spark gap near a magnetic pole. But we can at any rate rule out those theories which allow only one direction of drift, or those in which the direction was regulated by the aspect of the rotating star as seen by the observer.

The physics of the emission process is not revealed by the drifting phenomenon, since this may occur in a region remote from the emitter. For example, drifting in a spark gap at the poles provides a lateral movement in a stream of particles, which then sweep across an emitting region which might be close to the velocity of light circle. There is some evidence in favour of this location for the emitter from the work on pulsar slowdown rates, which indicate that the pulsars stop emitting when the magnetic field in that region falls below a critical value. On present evidence, the behaviour of pulsars seems to be completely dominated by the strength of the magnetic field.

SEAMOUNTS are active or extinct conical or flat-topped ocean floor volcanoes with elevations of at least 1,000 m, the majority not rising above sea level. They occur throughout the world's oceans but are particularly associated with the Pacific where, according to Menard (*Experientia*, **15**, 205; 1959), there are some 10,000. Several hundred of the Pacific volcanoes form linear chains (the best-known of which is the Emperor-Hawaiian chain) which are widely believed to result from the motion of the Pacific plate above hot spots with roots in the mantle. Most seamounts, however, are apparently individual bodies located at random on the ocean floor, although they tend to lie in clusters or bands.

As long as the oceans were thought

Do seamounts form near ridges?

from Peter J. Smith

to be permanent, the points of origin of seamounts posed no particular problem; volcanoes erupted on the ocean floor and thereafter remained fixed in position. But moving sea floors have introduced an ambiguity. Leaving aside the small proportion of seamounts aligned in chains, did the rest originate at random locations by some intraplate volcanic process or did they erupt near oceanic ridges and spread away to cover the ocean floor? As the number of seamounts per unit area of ocean floor does not seem to increase

away from spreading ridges (which means that the density of seamounts is no greater in older than in younger sea floor), the random hypothesis seems the less likely; but this can hardly be said to be definitive evidence.

An obvious way of settling the matter would be to date both seamounts and the surrounding ocean floor; a seamount embedded in ocean floor of comparable age would then imply ridge formation whereas discrepant ages would imply random volcanism. Unfortunately, experiments along these lines have provided conflicting evidence. Fisher *et al.* (*Science*, **160**, 1106; 1968), for example, found that three seamounts near the East Pacific rise were apparently up to 40 Myr younger than the underlying crust. The age of the

crust was poorly defined at that time, however, and a more recent tectonic reconstruction of the region by Herron (*Geol. Soc. Amer. Bull.*, **83**, 1671; 1972) has reduced the seamount-ocean floor age difference to between 5 and 6 million years. An even closer age agreement was obtained by Ozima *et al.* (*Earth planet. Sci. Lett.*, **8**, 237; 1970) for three Pacific seamount dredge samples (two Cretaceous, one Pliocene). Dymond and Windom (*Earth planet. Sci. Lett.*, **4**, 47; 1968), on the other hand, found that although two seamounts resting on Cretaceous crust southwest of Hawaii were also Cretaceous, a third was less than 1 Myr old.

In an attempt to resolve such conflict, Clague and Dalrymple (*Geophys. Res. Lett.*, **2**, 305; 1975) have now dated four seamounts in the central North Pacific—two from the Hawaiian ridge (Wentworth Seamount and Necker Island) and two from the Musicians Seamounts (Rachmaninoff and Khatchaturian). The samples from Necker and Wentworth are, respectively, at least 60 and 40 Myr older than the adjacent Hawaiian bodies as predicted according to the hot spot-plate motion hypothesis, and thus conflict with the concept of a Hawaiian chain ageing progressively westward. Rather than taking these results to disprove the age progression, however, Clague and Dalrymple propose that Wentworth and the northern part of Necker are Cretaceous seamounts that predated the formation of the Hawaiian chain and later became incorporated within it. Indeed, on the basis of seamount densities within the Pacific in general and in the area surrounding (but excluding) the Hawaiian chain in particular, the incorporation of at least this number of older seamounts is to be expected.

Assuming that Wentworth and Necker are unrelated in origin to the main Hawaiian chain, therefore, it only remains to compare their ages with those of their respective areas of surrounding ocean floor. Unfortunately, this is not easily done, largely because of the lack of magnetic anomalies in the vicinity of the Hawaiian ridge. From available fossil and radiometric age data and by extrapolating magnetic data from further afield, Clague and Dalrymple nevertheless conclude that Necker Island and possibly Wentworth Seamount are only slightly younger than their underlying crusts; likewise, the Rachmaninoff and Khatchaturian Seamounts, and two other Pacific seamounts previously well dated by other workers. Thus although the data are few and one or two discrepant seamount-crust ages still remain (notably that of Dymond and Windom), Clague and Dalrymple propose that most Pacific seamounts did indeed form on or near the East Pacific rise. □

Cyclotrons of all shapes and sizes

from E. G. Michaelis

The Seventh International Cyclotron Conference was held in Zurich on August 18–22 at the Swiss Institute for Nuclear Research.

THOUGH advancing into middle age and occasionally overshadowed by its Gargantuan progeny the cyclotron continues to develop vigorously, and after a series of facelifts its original passport photograph still current in physics textbooks is anything but a true likeness. The art of cyclotron construction remains challenging and cyclotrons serve a wide spectrum of scientific disciplines. Its devotees are still growing in number and budgetary restrictions have not diminished their enthusiasm or their ingenuity.

The Swiss Institute for Nuclear Research (SIN) welcomed more than two hundred participants from twenty-one countries with Swiss hospitality, and the report of the successful commissioning of the world's most powerful isochronous cyclotron by the Institute was a major contribution to the conference. News of similar successes achieved with large machines in Vancouver and Indiana proved that present designers can meet almost any challenge in this field.

Most of the 90-odd cyclotrons forming the subject of the conference are isochronous and sector focusing. Like the classical cyclotron they operate at constant orbital frequency but maintain this to relativistic ion energies by a magnetic guidefield increasing with orbit radius. Edge focusing between sectors of higher and lower field provides the axial stability which is absent

in a cylindrically symmetrical, radially increasing field.

In Spiral Ridge machines the sectors are curved to increase axial focusing, and in Split Pole cyclotrons the magnet poles are restricted to the high-field regions to increase the azimuthal variation of the field. The largest machines of this kind, the 590 MeV proton ring at SIN and TRIUMF, and the 520 MeV H^- accelerator at Vancouver combine both features. The magnetic fields in sector-focusing cyclotrons are subject to close tolerances and the ions are accelerated rapidly by high electric fields in two or more accelerating gaps to minimise the effects of local instabilities. Since technology and computer-aided design have made it possible to meet these conditions cyclotrons have become ever more powerful and versatile, many permitting the acceleration of a wide range of ions to prescribed peak energies. While problems of orbit stability are therefore largely resolved, those of ion sources, injection and extraction still limit performance and received much attention at the conference.

The growing interest in heavy, multi-charged ions calls for new ion sources to avoid the present need for several accelerators in cascade with ion stripping to higher charge states between successive stages. Reviewing the field J. Arianer (IPN, Orsay) showed that electron beam sources furnish more intense beams of highly stripped ions than the conventional PIG source or present laser sources.

Large and complex sources cannot be mounted in a cyclotron, and polarised, H^- and heavy ions are usually injected after some pre-acceleration. Only large split-pole machines permit mid-plane injection while particles are axially injected and deflected into the mid-plane of smaller machines. Interesting progress in mid-plane trochoidal injection however was reported by W. van Kampen (Delft Technical University) 200 keV protons are made to precess along a sector edge towards the middle of the cyclotron, where a suitably perturbed field gradually centres the orbits.

Ion extraction becomes more difficult as the separation between successive turns decreases with increasing particle energy, but notable successes in the extraction of energetic ions were announced at the conference. H. Willax (SIN, Switzerland) reported that more than 90% of the 590 MeV protons in the SIN accelerator can be extracted thanks to an energy gain exceeding 2 MeV per turn. J. R. Richardson (TRIUMF, Vancouver) described the simultaneous extraction of two proton beams of different energies from the large H^- accelerator and CERN achieved an extraction efficiency ex-



A hundred years ago

The celebration of the fiftieth anniversary of the opening of the first railway between Stockport and Darlington is attracting the notice of the French papers. Baron Charles Dupin, who published his celebrated work on Great Britain in 1826, described to the Institute that locomotives could never move, owing to the weakness of their hold on the rails, and that the use of horses could not be dispensed with. Baron Charles Dupin's reputation was so great that the truth of the statement was taken for granted from *Nature*, **12**, 483; September 30, 1875.

THE trend towards simplifying ecosystem complexity for comparative purposes by the use of common energetic terms often causes confusion by obscuring basic principles. The 'standing crop' of an ecosystem is one such term which indicates how much energy there is in the system at any one time. But the total amount of, say, primary production in energy terms available may not be the same as the amount actually available to the herbivores which feed upon it. Thus the figure for standing crop may be a red herring to anyone wishing to determine whether food is limiting in the ecosystem. A rule of thumb guide to consumption of grassland vegetation by herbivores is that rarely is more than 10% of annual primary production actually eaten (Wiegert and Evans, in *Secondary productivity of terrestrial ecosystems*, 449 (edit. by K. Petruzewicz) (Polish Academy of Sciences, Warsaw, 1967)), and in many instances 2% is nearer the mark (Grodzinski, *Acta theriol.*, 16, 231; 1971). On the face of it, therefore, food would not seem to be a limiting factor to herbivore populations.

The fallacy in this argument is to equate primary production standing crop with food. Food is more than just energy, and for most large ungulates a constituent of major importance is crude protein. Bredon *et al.*, among others, concluded that when the crude protein content of forage dropped to less than 4% dry weight, plant material ceased to function as food and ungulates feeding on it started to lose weight (*J. agric. Sci. Camb.*, 61, 101; 1963). Death does

not follow immediately and cattle kept on low quality grass continue to feed, but they face mounting physiological stress.

A careful demonstration of the hazards inherent in the standing crop approach to ecosystem dynamics is provided by Sinclair's recently re-

Standing crop \neq food

from our *Animal Ecology Correspondent*

ported studies on limiting factors in the Serengeti (*J. Anim. Ecol.*, 44, 497; 1975). He points out that herbivores—ungulates, small mammals and grasshoppers—consume 27%, 38% and 14% of the annual primary production of tall grassland, short grassland and kopje country respectively. This leaves a large portion of the annual production for detritivores and for fuelling fires. Why is it not used to support higher populations of herbivores? The answer is that for a part of the year it is unavailable as food, much of the protein and carbohydrate in the leaves having returned to the roots. Sinclair's figures show that from a spring level of 8% crude protein, dry season grass contains between only 1% and 3%. In the tall grassland, food required by herbivores is in excess of that available for the duration of the dry period—July to September. This is associated with a drop in small mammal and grasshopper populations and hence foraging pressure, but is compensated for by an increased intake by ungulates. Monitoring bone

marrow fat levels in wildebeest over the year reveals that they fall slightly during the dry season indicating some degree of physiological stress experienced at this time. In the kopje country where ungulates were seldom seen, herbivore food requirements outstripped production for a much shorter period largely because of the rapid decline in rodent and insect numbers. This strategy for accommodation to sudden unavailability of food is open only to small herbivores with fast growth and reproductive rates and is barred to ungulates who must tighten their belts and just make do.

Hairston *et al.* and other workers have suggested that since herbivore populations consume so little of the primary production they cannot be limited by lack of food, and so must be limited by parasites, predators or disease (*Am. Nat.*, 94, 421; 1960). The available evidence simply does not support this hypothesis. Sinclair is right to point out that an important implication of Hairston's hypothesis is that interspecific competition and niche diversification is precluded. There is no evidence that this occurs and plenty that it does not. Furthermore, consideration of mean annual values hides fluctuations not only in crude protein content but perhaps also in trace elements and other specific nutrients at certain times of the year. Availability of primary production as food is also affected by the physical correlates of feeding; crushing, despoiling and dropping. It is also affected by the nutritional and other needs of different age and sex classes in the populations.

ceeding 70% in its reconstructed synchro-cyclotron.

Work on heavy-ion acceleration was reported from several laboratories. A 25 MeV Tandem Van-de-Graaff under construction at Oak Ridge National Laboratory will inject ions into an existing cyclotron and will raise its energy to 800 MeV for ions of mass 160. M Gouttefangeas (CEA, Saclay) described a recently authorised French project in which three cyclotrons in cascade will accelerate the heaviest nuclei to 10 MeV per nucleon.

The large, split-pole cyclotrons favoured at present make for easy acceleration and extraction but require much steel and expensive buildings. Much more compact constructions can be achieved by using superconducting coils to provide fields of order 5T and the conference learnt of projects for such "superconducting" cyclotrons at the Lawrence Berkeley Laboratory and at Chalk River, Canada. Michigan State University is constructing a super-

conducting cyclotron magnet in order to study the problems posed by this new technique.

A tantalising project for a 100 mA 800 MeV proton cyclotron was described by Yu N. Denisov (JINR, Dubna, USSR), but its estimated radio-frequency power of 76 MW made it seem somewhat unrealistic.

Although primarily a gathering of accelerator physicists and engineers the conference devoted several sessions to the applications of cyclotrons and the range of topics proved the cyclotron to be a maid-of-all-work. The measurement by surface activation of abrasion in slurry-carrying ducts or of railway wheels, the determination of the fluorine content of a cup of tea by charged particle activation analysis, the study of nuclear level structures and excitation functions, radiation damage in reactor materials, the production of isotopes for medicine, neutron therapy, proton radiography and surgery are only a few of the topics covered by

various contributions.

M. M. Kligerman (University of New Mexico) reported on a comparative test of a treatment of skin melanoma by pions and X rays. The dramatic superiority of pions is a challenge to accelerator designers. A panel discussion on "Accelerators for Hospitals" reflected the difficulties, but tended to favour a proton-accelerator of variable energy.

The conference participants were able to visit the cyclotrons and experimental installations of SIN where a biomedical pion-channel and an eight-metre superconducting solenoid providing record fluxes of muons were of particular interest.

Professor M S Livingston, the co-inventor of the cyclotron, reviewed its early history in a delightful and informative after-dinner speech. Dr R Wideroe, the inventor of resonant acceleration also attended the conference and one may hope that the two veterans were pleased with the development of their brain-child. □

Satellites to the fore

from W. H. McCrea

EARTH'S satellite the Moon is not only the astronomical body other than the Earth about which most is known, it is also the astronomical body about which knowledge has increased most rapidly in recent years—thanks to the spectacular successes of the Apollo and Luna missions. Even more recently, Pioneer 10 and 11 have provided invaluable new information about the Galilean satellites of Jupiter. At the present time, therefore, astronomers are more satellite-minded than ever before. Above all, they are interested in the origin of satellites because this may provide a check upon, or even a clue to, the origin of the planets. After all, astronomers have only the one planetary system to study, whereas they have six satellite systems; so it is reasonable to think that the satellite problem might be solved before the planet problem.

T. Gold has reviewed the situation and made a number of suggestions (*Icarus*, **25**, 489–491; 1975). He begins by asserting that all planets for which satellite orbits would be stable do possess satellites, claiming that for Mercury the solar perturbation is too large, that the retrograde spin of Venus would cause satellites to spiral into the planet through tidal friction (but I think he means that the slowness of the spin of Venus would render it retrograde as seen by any possible satellite), and not mentioning Pluto. Anyhow, Gold is obviously correct in remarking that satellites are a common feature of the Solar System, and this stimulates him to seek a process of formation of the widest possible applicability. In general terms, he appeals to the dynamics of grains in the presence of massive gravitating bodies (Sun and planets) and of a resisting medium (neutral and charged particles and photons). If initially a lot of grains are moving at random in a gravitational field under the action of no other forces, the motion remains random, that is, it is conservative, and there is no tendency for the motion or space-distribution to become systematized in any way. But matters are different if dissipative forces play a part. If there are inelastic collisions between the grains themselves, then there may be focusing into the 'accretion streams' or 'jet streams' considered by some cosmogonists to be essential for the development of the Solar System. If there is a dissipative frictional drag exerted by a diffuse medium in the system, even at a stage when interaction between the grains is unimportant, there may be accumulation of grains in resonant bands in the

gravitational field of the moving planets. Both types of behaviour may be included under the heading of gravitational focusing. The novelty of Gold's discussion is to suggest a crucial cosmogonic role for the second type.

In bald terms the notion is: In the 'early solar nebula' grains were plentiful from direct condensation and from collisions between larger bodies of material. Under the action of the general gravitational field and the 'drag' forces, a grain near the central plane would normally spiral inwards or outwards from the Sun. Such spiralling could, however, be halted or speeded up if the motion became resonant with that of an orbiting planet. Thus lanes of accumulation or avoidance would have been formed. Here Gold remarks in passing that a lane of accumulation could be favourable for 'snowballing'; he suggests this as an explanation of the asteroids in Trojan orbits. He remarks too that in regard to any particular lane at any particular epoch there could be selection with respect to grain size or grain composition.

The main suggestion is that, material having been concentrated into a well-defined lane by resonance effects, when the resonance was broken, such material continued in a well-defined stream though once more in spiral motion; further, when it encountered a planet, there was a significant probability of its being captured into a ring round the planet. There would have been numerous cases of this happening throughout the system. Gold sees no serious difficulty in the passage from grains in circumplanetary rings to larger bodies, and the subsequent coalescence of these into one or more satellites round a planet as we now know them.

The interest of Gold's essay, one ventures to think, is its demonstration that the problem of the origin of satellites is still wide open. It was originally presented at a colloquium. It can scarcely have been intended to do more than throw out suggestions, for the treatment is non-quantitative throughout. This makes it difficult to comment further; however, when Gold writes, 'the step from circumplanetary rings to the formation of satellite bodies is not a difficult one and has been discussed on many occasions' one is bound to remark that although this has indeed been discussed often enough, one is not aware that it has ever been established that satellite bodies must form in these circumstances. Also, although there is much attractiveness in Gold's implication that all the regular satellites might have been formed by one and the same general process, the very great differences between the properties of the main satellites and the minor (regular)

satellites in the actual Solar System give strong indications of the operation of two distinct mechanisms of formation. Maybe we can look forward to a quantitative account of Gold's ideas in a form that could be more critically compared with observation. □

Nutrient balance during succession

from Peter D. Moore

THE developmental growth of an ecosystem, commonly termed succession, involves a steadily increasing biomass, reaching a maximum at the climax, or steady state, which is ultimately attained. The increase in living material during succession places a demand upon the inorganic, abiotic component of the system for those elements necessary for the construction of living tissues. Ultimately the biotic component thus becomes an important reservoir of nutrients—a feature which may contribute very considerably to the overall nutrient capital of an ecosystem (see *Nature*, **254**, 184; 1975). Actual data concerning the changing nutrient demand of the biotic component of an ecosystem during its development are, however, sadly lacking.

Experimental manipulation of watersheds in the Hubbard Brook Experimental Forest in New Hampshire, has provided a certain amount of information on which to speculate. Six small, undisturbed watersheds at this site have been studied intensively over a number of years and data concerning nutrient input from rainfall and output from stream drainage has been collected. In 1965 one of the forested watersheds (15.6 ha in area) was clear-felled and the resulting changes in the chemistry of the runoff waters were subsequently reported by Bormann, Likens, Fisher and Pierce (*Science*, **159**, 882; 1968). One of the most striking changes observed was the increased loss of nitrate nitrogen from the ecosystem following felling; a loss of $1.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ occurred in a control forested watershed, whilst $58.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ were lost from the clear-felled watershed. Many cations also increased in concentration in the runoff water, thus permitting the conclusion that disturbance led to increased leaching and therefore a reduced capacity for nutrient conservation.

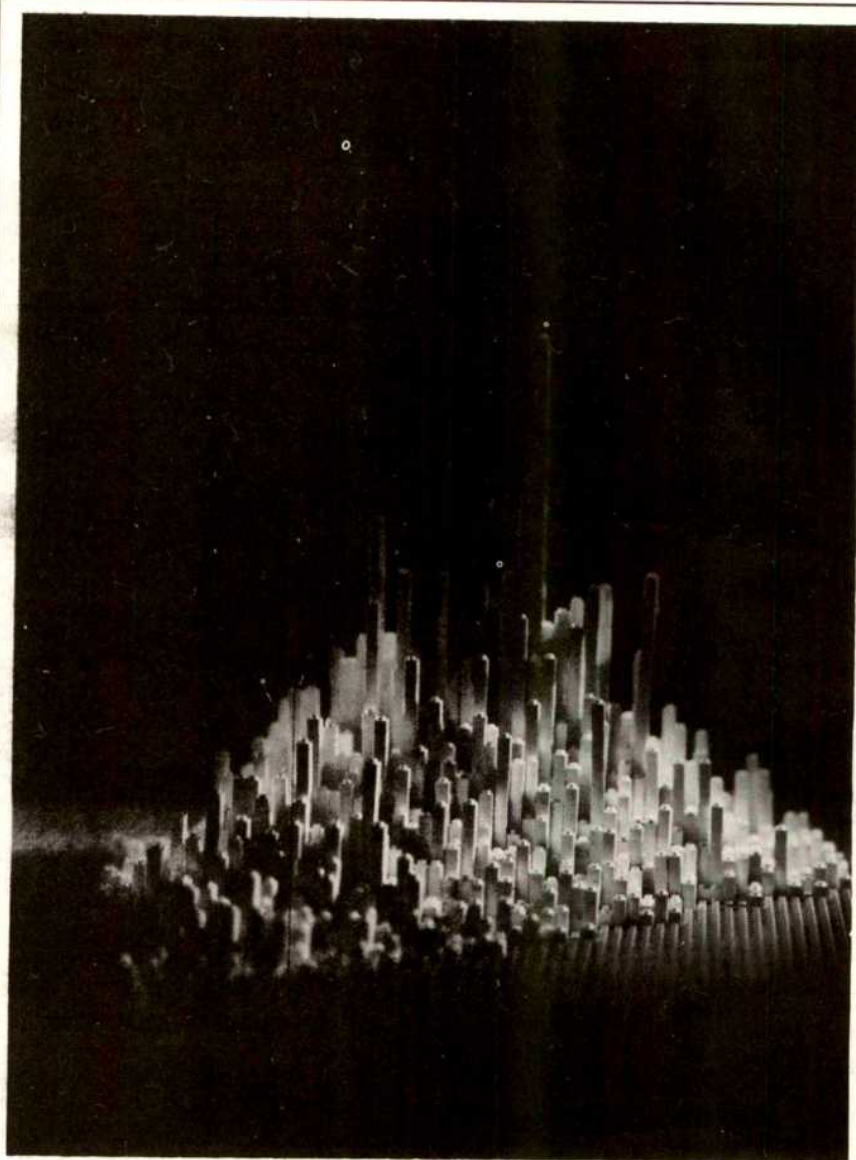
Marks and Bormann (*Science*, **176**, 914; 1972) further demonstrated that revegetation of a disturbed area led to considerably diminished nutrient runoff and they concluded that the early stages of succession which were then

occurring placed a high nutrient demand upon the abiotic environment as the biomass was in a rapid growth phase.

A rather different approach to the problem of documenting the relationship between the stage of ecosystem development and its ability to retain nutrients has recently been made by Leak and Martin (*USDA For. Serv. Res. Note*, NE-211; 1975). They have examined six forested watersheds in the north-eastern United States and have attempted to determine the approximate time when they were last disturbed. Obviously this is a complex problem, since different stands of trees within a stream catchment will have had different histories. A number of stands (between 7 and 41) were sampled within each catchment and such characteristics as the evenness of age within the stand and the proportion of tree species normally favoured by disturbances were noted. In this way it was possible to rank the six sites according to their 'weighted average age'. The time which had passed since general disturbance within the study sites was found to vary between 65 and 200 years.

Streamwater concentrations of nitrate from these watersheds had previously been monitored and these bear an evident relationship to the age since cutting. The site with an average tree age of 65 yr had stream nitrate concentrations of 2.3 p.p.m. (compare recently clearcut areas with 10.3 p.p.m.). Sites with age 75–110 yr trees had 0.15 p.p.m. nitrate in the streamwater, those of 125 yr had 0.8 p.p.m. and those of 200 yr, 2.2–4.8 p.p.m. Similar results have apparently been obtained by Vitousek and Reiners (*Bioscience*, in the press) at Mt Moosilauke.

The evident implication of these data is that low nutrient discharge is a feature of relatively immature ecosystems and that these systems become more leaky on attaining maturity. Nitrogen is a particularly complex element to choose for this type of survey. Not only is its input to an ecosystem complicated by biological fixation, but its output occurs as denitrification and ammonia flux to the atmosphere (see Denmead, Simpson and Freney, *Science*, **185**, 609; 1974) as well as by stream discharge. It is reasonable to assume, however, that as the steady-state ecosystem is approached, the total inputs of the element should be equalled by outputs and this equilibration may well account for Leak and Martin's observed increase in stream nitrate discharge with stand maturity. We may have to wait for a couple of centuries before these suggestions can be confirmed by the controlled experimental techniques of the Hubbard Brook team. □



This Manhattan skyline is a three-dimensional representation of the data from a recent search for charmed particles. A 3.6 GeV/c antiproton beam was used to investigate the interaction $\bar{p}p \rightarrow \bar{K}^0 K^+ \pi^- \pi^- \pi^0$ and blocks are mounted vertically to count mass values of $(\bar{K}^0 \pi^+ \pi^-)$ against the mass

values of $(K^+ \pi^-)$ of which there are two possible combinations giving the x and y horizontal axes. Peaks are evidence for the existence of charmed particles but those that are seen in these results do not tower sufficiently above their surroundings to be convincing—*CERN Courier* (15, 1975).

Solar flares and weather

from John Gribbin

IN *Nature* recently Olson *et al.* (257, 113; 1975) summarised evidence for a link between solar flares and changes in both the geomagnetic index and atmospheric vorticity on Earth. They ended their paper by saying that "we should examine the position of the flare on the disk of the Sun as a factor" in determining the response of weather systems to specific solar events, and in this context a brief communication just published in *Astrophys. space Sci.*, **35**,

L33–L34; 1975, is of topical interest.

In that paper J-T. Horng (Radio Physics Group, Telecommunications Laboratory, Chung-Li, Taiwan) reports a study of the association of geomagnetic events with complex sunspot groups, covering the period 1968–1972. Horng defines a complex sunspot group as one with two or more spots occurring in one penumbra, and has looked only at large geomagnetic disturbances ($A_p \geq 28$). The data include 36 such disturbances associated with 62 visible complete spot groups, and three geomagnetic events for which bad weather prevented any sunspot observations being made. It emerges that there is a

definite association between complex spots which occur between 6.7 and 19.9°E on the solar disk and the occurrence of large geomagnetic disturbances on Earth. The mean displacement of these spot groups, 13.3°E, can be taken as indicating that the spot groups and flares which cause the geomagnetic disturbances are located one day East of the solar meridian, allowing for their usual movement from East to West. For this particular sample, Horng also points out that 59.3% of the events occurred in the solar Northern Hemisphere below latitude 29°N and 40.3% in the Southern Hemisphere below latitude 25°S. But that is hardly surprising since the entire period of the study was close to the most recent solar maximum, when sunspots always form at low latitudes. □

Image processing at Stanford

from Albert Macovski

A meeting on "Image Processing for 2-D and 3-D Reconstruction from Projections: Theory and Practice in Medicine and the Physical Sciences", sponsored by Stanford University Institute for Electronics in Medicine and the Optical Society of America was held at Stanford University on August 4-7.

DUE primarily to the phenomenal success of the EMI brain scanner, the general subject of reconstruction from projections has generated a great deal of interest and enthusiasm. This particular meeting at Stanford, California is the second one of its type, the first taking place last year at the Brookhaven Laboratories.

Although X-ray and γ -ray imaging continue to dominate the scene, a number of novel application areas were presented which use comparable techniques. T. F. Budinger *et al.* (Lawrence Berkeley Laboratory) showed some preliminary results using heavy charged particles rather than X rays. Although the instrumentation is quite extensive, a significant dose reduction is obtained. J. F. Claerbout and P. S. Schultz (Stanford University) showed the application of similar techniques for seismic imaging using the reflections from sonic pulses. A cross-sectional image of the Earth is thus created to aid in activities such as oil exploration. J. F. Greenleaf, *et al.* (Mayo Foundation, Rochester) showed some exciting images of an excised dog's heart made by measuring the time-of-flight of ultrasonic pulses taken at many angles and positions. The reconstructed image

is a cross section representing sonic velocity. A number of other interesting papers were presented on non-radiographic applications including nuclear magnetic resonance zengmatography, electron microscopy, radio-astronomy and plasma diagnosis.

Many papers were devoted to the mathematics of reconstruction, both basic and related to specific applications. There was a strong trend, compared with the Brookhaven Conference, toward the use of direct as compared with iterative reconstruction procedures. In the basic area, R. P. Tewarson (SUNY, Stony Brook, New York) delighted the audience with his very profound fundamental treatment, well sprinkled with humour. He concentrated on methods for reconstructing the difference between the desired image and a standard image which he alluded to as the "average caucasian face." G. T. Herman *et al.* (SUNY, Buffalo), one of the pioneers of the iterative techniques, showed how these techniques converge to the least squares solution. Similarly P. R. Smith (Universitat Basel) along with T. M. Peters *et al.* (Christchurch Hospital, New Zealand) presented a fundamental exposition of the Fourier and back projection methods and the various aliasing considerations. M. Ein-Gal *et al.* (Stanford University) presented a decomposition of the projection data into consistent and inconsistent data and defined a quality factor representing their ratio. This talk invoked a lively discussion between the author, G. T. Herman and R. B. Marr of the Brookhaven Laboratory on the definition of inconsistency. O. J. Tretiak (Drexel Institute) presented an analysis of the point-spread function for direct reconstruction systems. This helped explain many of the artefacts which are being experienced.

In the area of mathematics related to specific applications a few investigators, including S. A. Johnson *et al.* (Mayo Foundation) discussed the problem of bending of rays due to refraction when reconstructions are made using optical or sonic waves. R. Gordon (National Institutes of Health), a pioneer in this field and the organiser of the meeting, presented a novel reconstruction system related to position reconstruction systems. Each event, as received, is assigned its most probable spatial position based on the previous events. A number of papers were presented on optimum reconstructions in the presence of incomplete data. L. R. D'Addario of the National Radio Astronomy Observatory and S. J. Wernecke (Stanford University) each presented papers on maximum entropy reconstructions. These gave impressive results in applications such as radio-astronomy and electron microscopy,

where the acquired data are sparse. A novel Fourier reconstruction system for nuclear medicine was presented by L. T. Chang *et al.* (Lawrence Berkeley Laboratory). A sequence of pinhole images were back projected and filtered in Fourier space to provide the desired reconstruction.

In the areas of improved X-ray and γ -ray reconstructions, the positron ring camera using coincidence techniques appeared quite exciting. L. Eriksson *et al.* (University of California, Los Angeles) and C. J. Thomson *et al.* (Montreal Neurological Institute) showed promising results. A number of papers were given on the problems of polychromatic effects in X-ray reconstruction. A. Macovski *et al.* (Stanford University) illustrated the nature of the distortion and a method of correction. R. E. Alvarez and Macovski (Stanford) showed how the X-ray spectrum could be decomposed so as to delineate density and atomic number effects in the reconstructed image. A similar decomposition based on chemical elements was presented by M. J. Berggren *et al.* (Mayo Foundation). The fan beam configuration which allows simultaneous acquisition of Earth projection received considerable attention. D. P. Boyd (Stanford University) described a fan beam instrument using a xenon detector and P. Stonestrom *et al.* (Stanford University) discussed the scatter problems. The fan beam geometry presents some interesting mathematical reconstruction problems which were treated by R. King (General Electric, Schenectady) and L. Wang and L. H. Cho (University of California, Los Angeles). □

Phonon scattering in solids

from a Correspondent

A conference on "Phonon Scattering in Solids" was held in Nottingham, UK on August 27-30. The proceedings will be published by Plenum Press.

At a good conference one finds out where the ragged ends in our knowledge lie. The conference recently held in Nottingham was no exception as contradictions were exposed by the close proximity of presented papers, and private conversations removed some of the gloss from published articles. But there were plenty of good new experiments reported which stimulated a lot of thought and the new techniques presented make it now possible to work at higher frequencies and with better spectral resolution.

Spectroscopy using phonons rapidly developed from the days of specific heat and simple thermal conductivity measurements when it became clear that there are resonant scattering processes which scatter phonons of well defined frequencies. Systems such as some magnetic ions in crystals, low-lying states in amorphous materials, copper pairs in superconductors, and charge carriers in semiconductors are strongly coupled to phonons and in many cases can only be studied by their interaction with injected phonons.

The two subjects which generated most enthusiastic discussion were predictably the Kapitza problem and amorphous materials. Heat transfer between two different solids, one of which at least is electrically insulating to stop electronic conduction, can be quite reasonably explained in terms of phonons propagating through the interface according to the classical ideas of elastic waves at interfaces as long as it is recognised that the material at the boundary may be somewhat different to the bulk. The way heat transfers from a solid to liquid or solid helium still remains a mystery however, even though the problem is being tackled with a variety of penetrating experiments. There is now little doubt that some of the phonons ($\sim 10\%$) go from the solid to the helium according to continuum theory so that $q_{||}$ is conserved and that the majority of the energy is carried by phonons which inelastically scatter at the interface. It is also clear that these inelastic processes are associated with the first few atomic layers of helium but the nature of these interface states is only speculative. Candidates such as surface waves, desorbed atoms and low energy rotons were hinted at but there was an understandable reluctance for necks to be stuck out too far after 34 years of looking for an answer.

Amorphous materials are in much the same state. Very elegant experiments were reported which indicate that there is a two- or three-level system which is strongly coupled to phonons and photons but whose nature is unknown. This situation is commonplace in high energy physics but in the solid state it is rare and so emphasises how sophisticated our knowledge of crystals really is. It is apparent that the amorphous states have a whole spectrum of relaxation times both to the lattice and to each other. In essentially d.c. experiments such as specific heat, all the states can absorb energy while in dynamic experiments with ultrasound and thermal conductivity only the states strongly coupled to the lattice phonons are seen. Even these states may not be seen if their relaxation time is too long or the ultrasound power too high, as they become saturated and then not

able to scatter. This last effect has been used to measure the time for interchange of energy between states by hole-burning techniques with ultrasound in a similar way to that used in electron paramagnetic resonance for inhomogeneously broadened lines.

A great deal of work is going into magnetic ions which are strongly coupled to the lattice. Many mysteries remain here but one gets the feeling that the subject is bedevilled by samples. There are always too many resonances than can be accounted for but this is not surprising as very dilute impurities can have a large effect and strains as small as 10^{-6} can shift the energy levels. Even the strains due to the dopant being studied are a problem. When positive identifications can be made however, theory seems largely able to cope.

Phonons from superconducting tunnel junctions are being used increasingly to study all those systems with frequencies up to 1 THz. Frequencies an order of magnitude higher were claimed to be generated by shining an infrared laser on to quartz, however these phonons were not measured directly as they were detected bolometrically. It is not clear why this method works but no doubt much more work will be done on this system. Optical Brillouin and X-ray scattering are also being used in complicated experiments to study phonons.

The many other topics discussed at the conference can be read about in the proceedings which it is hoped will be published by the end of the year. There were some 110 papers which indicates the growing enthusiasm for this subject. \square

European astronomy at Leicester

from A. C. Fabian

The first European Conference on Astronomy was held at Leicester, UK on August 12-14.

THE first European Conference on Astronomy recently attracted about 250 astronomers to the hot lecture theatres of Leicester University. At least four orbiting satellites carry European X-ray instrumentation and these provided many of the new results. Radioastronomy was well represented, but there seemed surprisingly little from optical observers.

Transient X-ray sources are the most exciting objects to have been discovered by the Ariel V satellite. Several had been known before the launch of Ariel V last year, but few could have

predicted the large number of bright ones that have now been observed. Estimates seem to indicate one bright transient every 10 days or so, and K. A. Pounds' group at Leicester gave day by day reports on the latest (A0621-00), which is now the brightest X-ray object in the sky. It was speculated that this new source is similar to the binary X-ray sources, and may be associated with accretion on to a white dwarf, neutron star or black hole. This implies a distance less than a kiloparsec or so if the object is not to exceed the Eddington limit for a solar mass object. Above this limit radiation pressure will seriously modify the accretion flow and the result would probably prevent detection of the source. Extrapolation from known X-ray sources would suggest that the optical companion should be visible between about eighth and twelfth magnitude, at most. A better position, to an accuracy of a few arc min should be available and allow for an identification now that the modulation collimators on Ariel V and the American SAS 3 have been pointed in that direction. Spectral changes show a softening of the spectrum as the intensity increased. H. Bradt (Massachusetts Institute of Technology) reported that no periodicities between 0.8 s and 2,000 s were detectable in preliminary data from SAS 3.

Pulsations in the minute range are well known since the discovery of Ariel 1118-61 last December, as recalled by J. G. Ives (Mullard Space Science Laboratory, University College, London). Bradt showed the X-ray "light" curve of A0536+26, a transient source discovered by Ariel V earlier this year near the Crab nebula. A regular dip every 104 s at high energies (>10 keV) degenerates into about six structured dips below about 5 keV. Pulsations of the more constant source, Cen X-3, were shown by I. R. Tuohy (MSSL). He finds a broad double-peaked pulse from the Ariel V data unlike the single pulse that was usually observed from Uhuru. Observations of pulses from Cyg X-1 were reported by G. Auriemma (Frascati). This is remarkable because the pulses, with periods of about 0.083 s were found optically. They represent a significant fraction ($\sim 5\%$) of the optical flux from the system. Only a non-thermal mechanism can radiate so much energy from so small a region.

The recent series of occultations of the Crab nebula, which contains a 33 ms pulsar and was the site of SN1054, have been well covered by X-ray detectors carried on balloons and rockets. F. D. Seward (Lawrence Livermore Laboratory) showed the results of a rocket observation made last November. The moon started occulting the X-ray source soon after

the detectors were locked on, and the signal gradually decayed over the next 150 s or so. Near the end of the flight the detectors were displaced about 10° to take a background reading. A significant drop in count rate was immediately recorded. Seward and his colleagues suggest that this was due to a diffuse source associated with the unoccluded part of the nebula. At a temperature $kT \sim 0.8$ keV and luminosity $\sim 10^{38}$ ergs s^{-1} , this puts at least part of the Crab in line with other X-ray supernova remnants.

Very long baseline interferometry radio observations of the nucleus of NGC1275 (3C84) by E. Preuss (Bonn) and his colleagues contrasted well with the maps shown by A. G. Willis (Leiden) of the large radio galaxies observed from Westerbork. The VLBI observations could be interpreted in terms of a stable map showing 10 components on the scale of ~ 1 milliarc second! (This is about the angle subtended by a man on the moon.) At the other extreme, the large scale emission regions of 3C236 and DA240 could accommodate many Cygnus-As. A compact, highly polarised, and unresolved source has been found at the leading edge of the eastern component of 3C236 which, if not a background source, will be of interest in discriminating between the many theories of radio galaxies.

Many more new and unpublished results and ideas were presented at this very successful conference. In his review of the theory of supernova remnants, S. Gull (Cambridge), showed a memorable radio "photograph" of the supernova remnant Cas A "taken" with the 5 km telescope. The turbulent detail in the shell of this remnant is as dramatic as any optical picture of the Orion nebula. Popular books will never look the same when these new pictures start replacing the old favourites.

The meeting showed that European astronomy is in a healthy state and it is to be hoped that the European Conference on Astronomy may become an annual event. \square

X rays and ion transport in liquids

from Andrew Holmes-Siedle

THE use of photographic film to record X rays has always had the disadvantage that one has to wait to see the image. Another less obvious disadvantage is the "chemical fogging" which impedes perception of fine detail. Direct viewing of fluorescent screens demands high doses to the subject or the use of intensifying systems which, though

costly, do not always give enough detail and do not yield a record of the image in "hard copy". There is now growing interest in a method which seems to combine the virtues of each system with cheapness and simplicity and involves some interesting physics. At least two groups are working on it, using the names 'electron radiography' in the USA and 'ionography' in the UK.

The X-ray shadowgraph image is converted into a charge image on a dielectric by the ionisation of a gas or liquid in a chamber and the transport of the positive ions in a straight line to one wall of that chamber, where they collect as stored charge on a dielectric sheet. This charge image can then be developed with a powder in the same way as in the well-known xerographic processes. The transport of the ions is effected by a fairly high applied electric field and no pre-charging of the sheet is necessary. Such an idea may sound simple but it is only recently that a method yielding a satisfactory resolution has been developed.

Early attempts to use electrostatic image formation all involved the use of pre-charged insulator surfaces. Even before the use of film was established, within months of the discovery of X rays, Righi (*The Electrician*, 37, 75; 1896) had found that X rays would discharge ebonite and that the pattern could be made visible with powders. The discharging of a selenium plate by X rays was embodied in the original Xerox patents but, despite some investigation in the 1950s, "xeroradiography" has not been widely used. Criscuolo (*Non-Destructive Testing*, 14, 28-36; 1956) then found that the X-ray-induced conductivity of air over a charged insulator surface could be used to produce an image but that resolution was still poor, mainly because of the small fraction of photons colliding with gas near the plate. The step to a good image was made by increasing both the atomic number and density of atoms above the insulator and by replacing the field from the charged insulator by an applied field, then simply inserting an uncharged insulator foil between the field electrodes and allowing it to intercept and trap the charged species which drifted towards it. Two groups realised the efficiency of this method at about the same time. H. E. Johns, J. W. Boag and others reported favourable results in 1974 (*Br. J. Radiol.*, 47, 519-529) while A. P. Proudian and others presented a verbal description in 1972 and published results in 1974 (*Radiology*, 110, 667-671).

In both of these publications, the density of atoms near the insulator was increased by using a gas at pressure. Xenon at ten atmospheres proved ideal, since this element has an absorption

peak which nicely matches the white radiation spectrum emitted from a 100-keV X-ray tube. The polarity of field is normally such that the xenon ions bombard and charge the surface of the insulator foil. While the electrons will also produce a charge image, the British workers showed that scattering on the way to the foil caused serious loss of resolution. This team thus used the name ionography for the technique, while the American team preferred to call it electron radiography to avoid the historical associations of the former term.

The American team, Allan *et al.*, have now published (*J. appl. Phys.*, 46, 2766-2767; 1975) details of an improved process, termed "liquid-absorber electron radiography", which avoids the use of gas at high pressure. The extra simplicity of using a liquid transporter medium clearly confers engineering advantages but physicists should also be intrigued by this new requirement for efficient transport of charge in a condensed phase. The composition of the liquid will have to be tailored to meet several unusual demands. Not only must it be a good X-ray absorber but its nature must be such as to minimise recombination and scattering of the ions as they drift to the foil. Of course, in order to reduce lateral charge movement and "fog" from leakage between the field plates, the liquid must also be an excellent insulator when not being irradiated. Since it is desirable to sweep out the ions rapidly, fields near to those producing avalanche effects in the medium will be favoured. Since the science of charge transport in normally non-conducting materials in the condensed phase is still in its infancy, this new demand for know-how may give a welcome increase of interest in the basic processes of transport.

The prediction of the British workers, that a very thin pencil of X rays will produce a spot image of radius about 0.175 mm at half maximum in high-pressure xenon is nicely borne out in their experiments, which indicate an image resolution of about 10 line pairs per mm. The results quoted by the US workers for the liquid medium show a strong improvement on this result, the resolution for the latter medium being much better than 10 line pairs per mm. Clearly, charge image radiography has come of age but should still provide some interesting physical problems during its further maturation. \square

Erratum

In the article 'Jupiter XIII' (*Nature*, 257, 178; 1975) para 2, line 20 should read "magnitude about +22" not "magnitude about +0.22".

articles

A subharmonic lunar tide in the seas off Western Europe

D. E. Cartwright

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A small and often neglected tidal component, which is an exact subharmonic of the M_2 tide, is shown to have unusually large amplitudes over a sea area stretching from Shetland to the Azores, including the North Sea. An explanation is offered in terms of normal oceanic modes recently computed by Platzman, and implications for tidal prediction procedures are discussed.

THE usual picture of the lunar tide-generating potential, a symmetrical oval shape, concentric with the Earth and with its axis directed towards the Moon, is only approximately correct. Because of the finite distance of the Moon the oval is not quite symmetrical, in that the potential on the part of the Earth's surface nearest the Moon is about 1% less than that on the part farthest away. The asymmetry, illustrated in Fig. 1a, produces a daily tidal component which is an exact subharmonic of the principal lunar half-daily tide (M_2), the period of its cycle being a mean lunar day (I ignore the ter-diurnal distortion M_3). This tide is distinct from that normally thought of as the "diurnal tide", and which is due to the monthly excursions of the Moon above and below the Equator. That diurnal tide does not contain a component which is an exact subharmonic of M_2 . If the orbit of the Moon were permanently in the equatorial plane, as in Fig. 1a, there would be no diurnal tides of the usual sort (Fig. 1b), but the subharmonic lunar tide would remain.

The facts set out above are known in tidal theory, but the subharmonic effect is usually neglected in practice because the asymmetry in the generating potential is so small. What is not generally known is that at a number of places, representative of a wide sea area off western Europe, the subharmonic tide actually dominates that part of the spectrum known loosely as the " M_1 tidal constituent". The M_1 group of lines, as it is more properly called, consists of a cluster of harmonic components of the tide-generating potential within 1 or 2 cycles yr^{-1} of 1 cycle per lunar day. It may be split into two distinct groups, one associated with the function

$$\xi^2 P_2^1(\theta) = \xi^2 \frac{3}{2} \sin 2\theta$$

where ξ is the Moon's parallax, and θ is terrestrial colatitude, and the other with

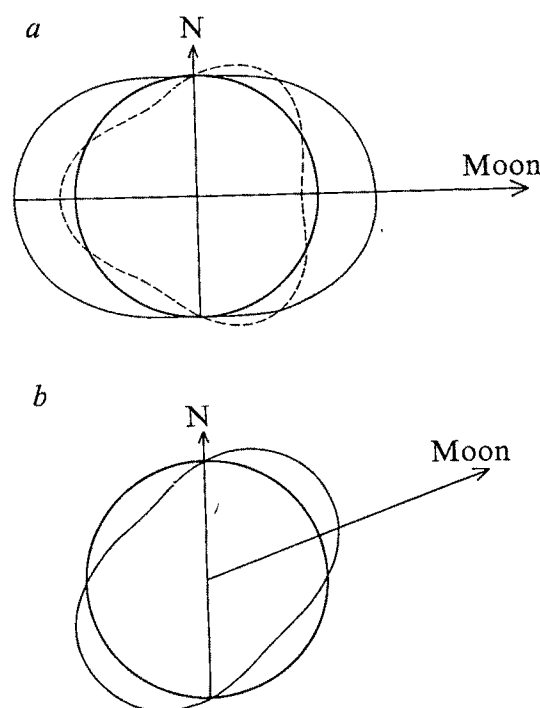
$$\xi^3 P_3^1(\theta) = \xi^3 \frac{3}{2} \sin \theta [(5 \cos^2 \theta) - 1]$$

(P_2^1 is represented in Fig. 1b, and P_3^1 as the broken curve in Fig. 1a). Their spectra are shown in Fig. 2a and b. Since ξ is of

the order $1/60$, the second group tends to have much smaller amplitudes than the first, and this is evident when comparing the logarithmic scales of their spectra. The other distinction is that the P_2^1 group is dominated by two lines at about $1/9$ cycles yr^{-1} on either side of the central frequency 1 cycle per lunar day whereas the P_3^1 group has essentially one strong line at just the central frequency. Figure 2c, d and e shows the corresponding part of the spectra of 18-yr sections of tide gauge records from Newlyn (two sections) and Brest. Lines corresponding to the M_1 group in the potential show up clearly above noise level, and the salient fact is that the 1 cycle per lunar day (subharmonic) line from P_3^1 is the largest of all at both places. This represents a magnification of the P_3^1 lines relative to the P_2^1 lines by a factor of more than 10.

A similar phenomenon had been observed¹ in the analysis of records from Simons Bay, South Africa covering nine years, but there the P_3^1 line dominated on account of an unusual node

Fig. 1 a, Cross section of components of gravitational potential with Moon over the Equator. —, M_2 component. - - - - -, Subharmonic M_1 component (magnified $\times 50$ relative to M_2). b, Form of principal diurnal component with Moon north of the Equator. In both cases, the circle represents the Earth's surface, and other curves are drawn relative to it.



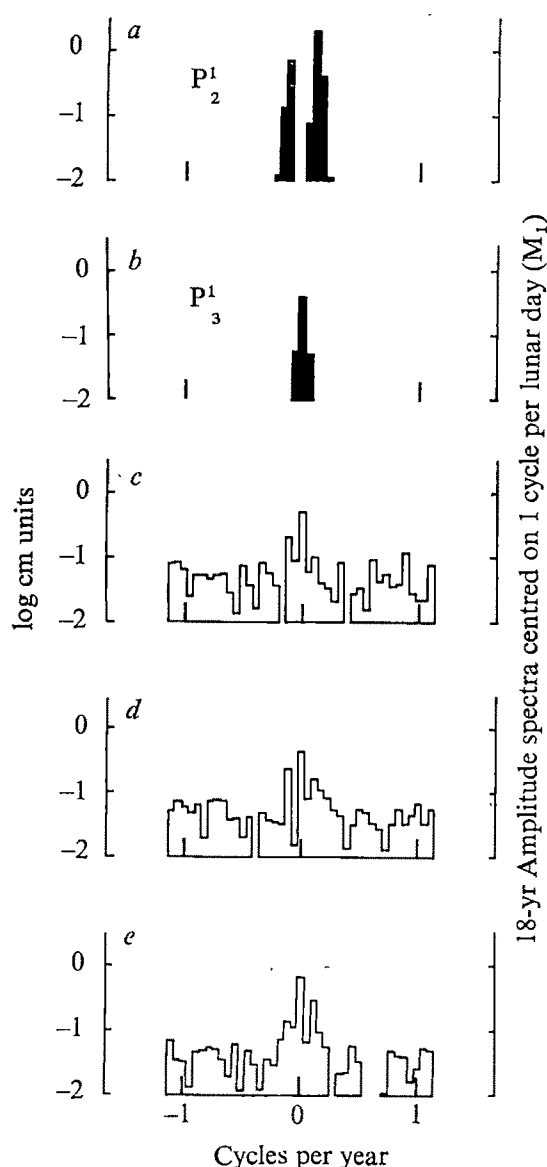


Fig. 2 Amplitude spectra at $1/18$ cycles yr^{-1} resolution near 1 cycle per lunar day. The base scale shows the heterodyned frequency. *a*, P_2^1 gravitational potential; *b*, P_3^1 gravitational potential; *c*, sea level at Newlyn, 1915-33; *d*, sea level at Newlyn, 1951-69; *e*, sea level at Brest, 1915-33.

in the principal diurnal tide at a frequency very close to 1 cycle per lunar day. From further enquiries, it seemed that the subharmonic M_1 tide, as we may call the principal component associated with the P_3^1 harmonic, was first identified in sea level records from Cuxhaven, around 1968 (W. Horn, personal communication). A phenomenon as widespread as Brest, Newlyn and Cuxhaven suggests an oceanic origin, so I searched for other European sea level records of sufficient length for its resolution. The line-separation of $1/9$ cycles yr^{-1} requires 9 yr of records for clear resolution, but 5-6 yr are adequate with special methods², if the noise level is low. Records from Southend covering 18.5 yr recently analysed for other objectives (M. Amin, to be published), was found to have a subharmonic M_1 tide similar to Cuxhaven. Shorter records from the Scilly Isles, Malin Head, Stornoway and Lerwick confirmed its dominance, as at Newlyn, over the entire oceanic coastline of Britain. Another short record, from Dunkerque, gave results similar to Southend.

The results from the places mentioned previously are shown in Table 1. H and G are the amplitude (mm) and Greenwich phase lag (degrees) of the named constituents, and R the amplitude ratio (admittance) to the gravitational potential, in the

normalisation of Cartwright and Tayler³. I have used M_1' to denote the subharmonic tide (in accordance with modern German practice⁴), and M_1 the principal component of the P_2^1 tide, which some⁴ prefer to call "NO₁". The principal lunar semidiurnal tide M_2 is included for comparison. The three entries for Newlyn are for three consecutive 18-yr spans of data, the spectra from two of which are included in Fig. 2; they show the order of variability which may be expected due to noise. There is no obvious relationship between the constants of M_1 , M_1' and M_2 , but the main features described above, large H and R for M_1 , small for M_1' , are clearly supported. One notices that the amplitude ratios for M_1 are about 1-2, which is not very large, but nevertheless comparable with the admittances at M_2 which are generally considered 'large' in the north-east Atlantic. The admittances for M_1' are, however, only of the order of 10^{-1} , which makes their H values small in comparison with those of M_1 . The dominance of M_1 in this area, then, is due not only to magnification of the P_3^1 tide but also to a depression of the common diurnal tide in the Atlantic Ocean as a whole.

The two island stations included at the bottom of Table 1 show that at the distance of the Azores, the dominance of M_1 is reduced to equality in H , whereas as far as Bermuda R become of similar order, and M_1' dominates. The result for Bermuda is, in fact the norm, confirmed at for example Honolulu (19 yr), Venice (12 yr), and Antarctica (9 yr). The phenomenon of a relatively magnified M_1 may well be confined to western European seas (and the vicinity of Simons Bay).

Heuristic explanation

Before proposing a physical cause for the enhanced M_1 or suppressed M_1' tide, it is worth dismissing some possible but implausible suggestions.

(1) Anomalous lines in tidal spectra are often due to nonlinear interactions at sum and difference frequencies. This can indeed affect M_1' , through interaction between N_2 and O_1 , but only noticeably at the shallow water ports of Cuxhaven and Southend. There are no tidal lines of any importance whose frequencies differ by that of M_1 (1 cycle per lunar day).

(2) One might envisage a nonlinear mechanism causing a subharmonic-harmonic instability between M_1 and M_2 . But this would suggest some correlation between the amplitudes of M_1 and M_2 , which is not observed (Table 1). At these places there is nothing anomalous also about the diurnal line K_1 , which is a subharmonic of K_2 .

(3) Some tides are amplified at the resonant frequency of a semi-enclosed basin, as in the Bay of Fundy. But a resonance which selects M_1 and not M_1' implies a tuning width of about 0.1 cycles yr^{-1} ; such fine tuning is unknown in oceanography.

My suggested explanation involves spatial matching of forcing function and normal modes of oscillation, rather than resonance in frequency. Figure 3*a* and *b* shows the wave form of a normal mode of the Atlantic-Indian Ocean system at a period of 23.5 h, computed by Platzman⁶. This is the nearest in frequency to the diurnal tides of many such modes, and can be expected to form an important contribution to the forced tidal wave at 1 cycle per lunar day. Concentrating on the Atlantic, which has the largest wave elevations, one sees an anti-clockwise amphidromic system in mid-northern latitudes and a similar clockwise system in mid-southern latitudes. The arrows indicate the direction of wave propagation at (arbitrarily) 0 hours. At that time in the North Atlantic there is a northbound wave crest with northward currents in the eastern sector, whereas to the west of the amphidrome there is a southbound wave-trough, which also has northwards currents. So at 0 h there are general northward currents in the North Atlantic, and similarly there are general southward currents in the South Atlantic. A quarter of a cycle later there will be generally westward currents in both North and South Atlantic; half a cycle after 0 h, southward currents in the North Atlantic, northward currents in the South Atlantic, and so on. The point is that this pattern of motion matches the rotary motion of the

Table 1 Positions of places analysed, and the amplitudes H (mm), phase lags G (degree), and admittances R of the subharmonic M_1 tide, of the nearby P_2^1 term (M_1), and of the M_2 tide

Place	Latitude	Longitude	M_1			M_1'			M_2			yr
			H	G	R	H	G	R	H	G	R	
Newlyn, Cornwall I	50° 06'N	05° 33'W	5.3	284	1.3	1.0	39	0.05	1,700	135	2.69	18
Newlyn, Cornwall II	50° 06'N	05° 33'W	4.6	274	1.1	1.1	26	0.05	1,704	135	2.70	18
Newlyn, Cornwall III	50° 06'N	05° 33'W	4.4	277	1.1	1.6	49	0.08	1,702	135	2.69	18
Scilly Isles, Cornwall	49° 55'N	06° 20'W	5.0	276	1.2	3.1	43	0.15	1,764	130	2.79	6.25
Brest, Brittany	48° 23'N	04° 30'W	6.9	268	1.7	3.0	31	0.15	2,401	109	3.23	18
Malin Head, Eire	55° 22'N	07° 20'W	8.9	312	2.2	5.6	101	0.27	1,061	183	1.68	8
Stornoway, Hebrides	58° 12'N	06° 23'W	8.8	308	2.2	6.7	81	0.32	1,363	198	2.16	6.5
Lerwick, Shetland	60° 09'N	01° 08'W	7.4	330	1.9	3.7	91	0.18	585	312	0.92	9
Southend, Essex	51° 31'N	00° 44'E	9.5	112	2.4	2.5	88	0.12	2,045	354	3.23	18.5
Dunkerque, France	51° 03'N	02° 22'E	9.2	104	2.3	6.6	101	0.32	2,103	353	3.30	5.25
Cuxhaven, Germany	53° 51'N	08° 44'E	9.2	214	2.3	(below noise)			1,322	344	2.09	23
Terceira, Azores	38° 40'N	27° 14'W	2.1	275	0.5	2.2	41	0.11	447	65	0.71	5.25
St Georges, Bermuda	32° 24'N	64° 42'W	1.0	260	0.3	3.4	191	0.16	357	358	0.57	9

P_3^1 forcing function, but mis-matches the motion of the P_2^1 forcing function.

The semicircular arrows in Fig. 3c and d represent the azimuth direction of the horizontal stress due to the P_3^1 (left) and P_2^1 (right) components of the Moon's gravitational potential, during a half cycle starting with Greenwich transit (tails of arrows). The direction of rotation reverses across certain latitudes which are drawn. In the wide zones between latitudes 26.5° and 59° the P_3^1 stress rotates in the same sense and phase as the two gyres of the Atlantic in Platzman's model, and with some allowance for change of longitude, the more northwards Indian Ocean gyre is matched also. The matching in the gyre below 60°S is not so good, but on the whole the work done on

this mode of motion by the P_3^1 stress, as measured by the scalar product (stress \times current) integrated over the whole area will be rather high. The P_2^1 stress pattern, on the other hand is much less effective, being northerly in both north and south (below 45°) at 0 h, then westerly in the north Atlantic and easterly in the south, quarter of a cycle later, and so on. The work integral for P_2^1 will be small, and one expects this stress pattern to generate rather weak tides, as observed.

Further support for the importance of Platzman's near-diurnal mode comes from the large amplitudes above latitude 45°N, shown by the numbered broken lines in Fig. 3. The observed amplitudes for M_1 are large in just this area, and do in fact increase towards the north. (The largest amplitudes of

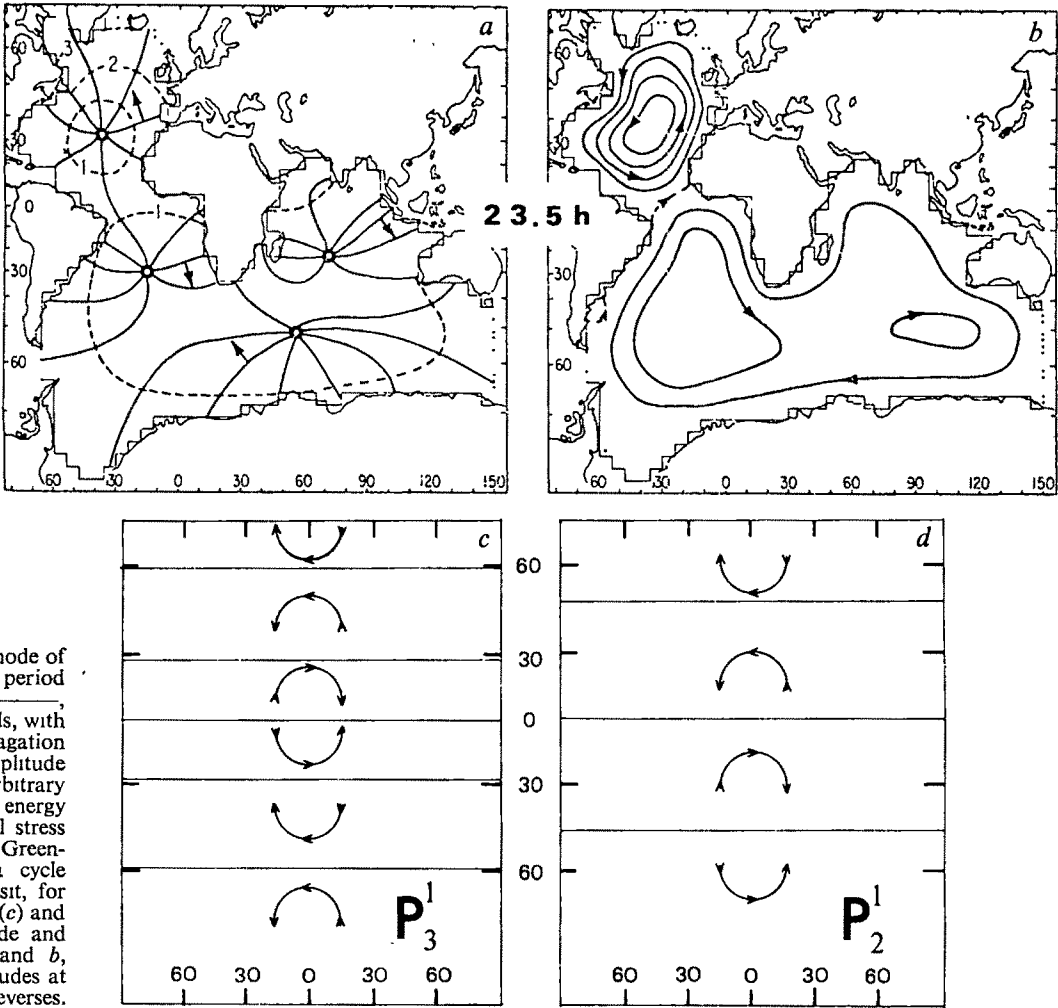


Fig. 3 a-b, Gravity wave mode of Atlantic/Indian Oceans at a period of 23.5 h (after ref. 5). a, —, Isophase lines at 45° intervals, with arrows in direction of propagation at 0°; - - - - - isoamplitude lines with amplitude in arbitrary units. b, stream function of energy flux. c-d, Direction of tidal stress (potential gradient), on the Greenwich meridian for half a cycle (180°) following lunar transit, for P_3^1 component of potential (c) and P_2^1 component (d). Latitude and longitude scales as in a and b, straight lines mark the latitudes at which sense of rotation reverses.

all, at Cuxhaven and Southend, may be regarded as a displaced product of the tides at the northern entrance to the North sea.) One would expect from Fig. 3 to find still larger amplitudes in north-east Canadian waters, but this has not been investigated. The accuracy of Platzman's model may in any case fall off near its northern boundary.

Implications for tidal prediction

The classical " M_1 tidal constituent" (my M'_1) has always fitted awkwardly into the conventions of "harmonic" prediction, on account of its two major spectral lines separated by 0.23 cycles yr^{-1} , which is too close for resolution from a year's analysis but rather far for an accurate 'slow modulation' scheme. Schureman⁷ devoted three pages to it; Rossiter⁶ included it in a discussion of "troublesome harmonic constituents" and referred to an epistolary dispute about M_1 between Horn and Doodson in 1947. The usual method, which ignores the possible existence of the subharmonic term, takes a nominal frequency of 1 cycle per lunar day ($14^\circ.492 \dots \text{h}^{-1}$) but adjusts with a modulating function whose phase advances from 0 to 360° every 9 yr. Even the definition of the modulating function differs according to authority^{7,8}, so that published constants are valid only if used by the method of the authority which produced them. This odd situation has survived over the years only because the constituent is, after all, rather small, so that errors in prediction arising from it will not be too serious.

The identification of the subharmonic M_1 in the sea area specified confuses the issue still further, and a reappraisal of the whole situation is needed. It is worth mentioning three logical solutions to the difficulty.

(1) One may treat M_1 and the two companion terms of M'_1 as three distinct constituents, each of which has a minor modulating function depending on the lunar 'node', as in common constituents. This is, in fact, the method suggested by the recent DHI tables⁴. To identify these constituents, however, requires several years of data to be analysed, typically 9 yr, or preferably 18 yr.

(2) The "response" method² may be used, which automatically treats P_2^1 , P_3^1 and nonlinear terms separately in an optimum way. This still requires a minimum of 5–6 yr of data to achieve the separation reliably.

(3) If one is restricted to the standard '1-yr analysis' a compromise must be made, depending on the sea area. In areas where the subharmonic M_1 tide has been shown to be normally small, the standard modulation scheme is adequate, although some unification of constants would be desirable. In north-west European seas, I have shown here that one would do best to treat M_1 as a pure subharmonic line, and either ignore M'_1 or infer it from continuity with the more reliable terms O_1 and K_1 from the P_2^1 tide.

I am grateful to Mr R J Tayler and Mrs Anne Edden for assistance with the analysis of the Newlyn data.

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Meanders and long waves in the equatorial Atlantic

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Observations from the GATE Equatorial Oceanographic Experiment are presented. They reveal large scale meandering of the westward flowing South Equatorial Current and of the eastward flowing Equatorial Undercurrent with time scales of 2–3 weeks. Meandering of the flow pattern was found to be related to corresponding displacements of the high salinity core of the undercurrent. The observations tend to support the assumption of a long wave propagating westward with a phase speed of 2.3 m s^{-1} and a wavelength of 3,200 km. A possible explanation may be given in terms of unstable waves caused by large scale horizontal shear in the Equatorial Current System.

THE Equatorial Experiment, one of the components of GATE (Atlantic Tropical Experiment of the Global Atmospheric Research Programme, see refs 1–3), was an oceanographic experiment designed to determine the spatial and temporal

scales of transient equatorial phenomena, to study their interaction with equatorial currents, and to investigate the dependence of upper ocean phenomena on atmospheric forcing. The platforms from which measurements were made included stationary and roving ships as well as an array of moored buoys carrying measuring equipment. This experiment culminated during phase II of GATE, from July 27 to August 16, 1974 (see Fig. 1). Complementary observations were made during phase I of GATE, which preceded this project, and phase III, which followed it. Here we report on preliminary results from the Equatorial Experiment and deal mainly with large scale meandering and wave phenomena of the currents within $\pm 50'$ latitude of the equator. Information on these phenomena is based on: (1) current profiling measurements from the research vessels Capricorne (France), A. Dohrn (FRG), A. V. Humboldt (GDR), C. Iselin (USA) and Ak. Kurchatov (USSR); (2) moored current meter arrays positioned by the research vessels Discovery (UK), A. Dohrn, Ak. Kurchatov, Passat (USSR) and Trident (USA); (3) measurements of the temperature and salinity fields from all vessels mentioned above as well as additional measurements from the RV Atlantis II (USA).

The observational techniques in categories (2) and (3) are usual oceanographic procedures, but the observations in category (1) constitute a novel approach (for details on the

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Fig. 1 Central observational array during the GATE Equatorial Experiment.

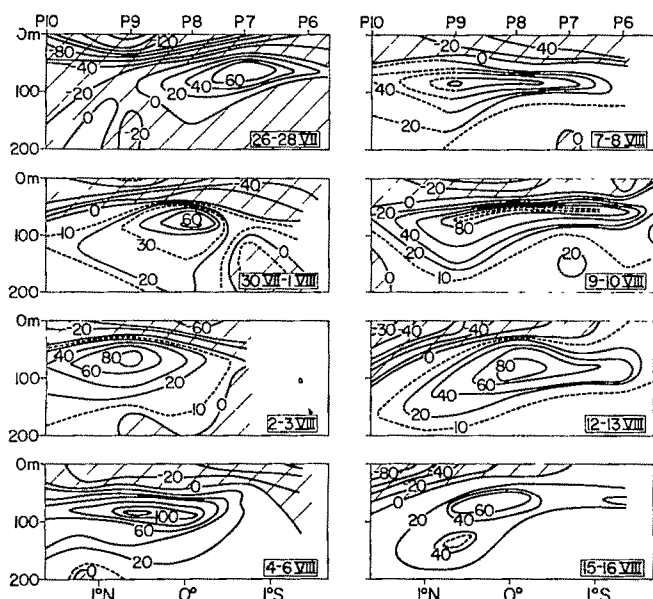
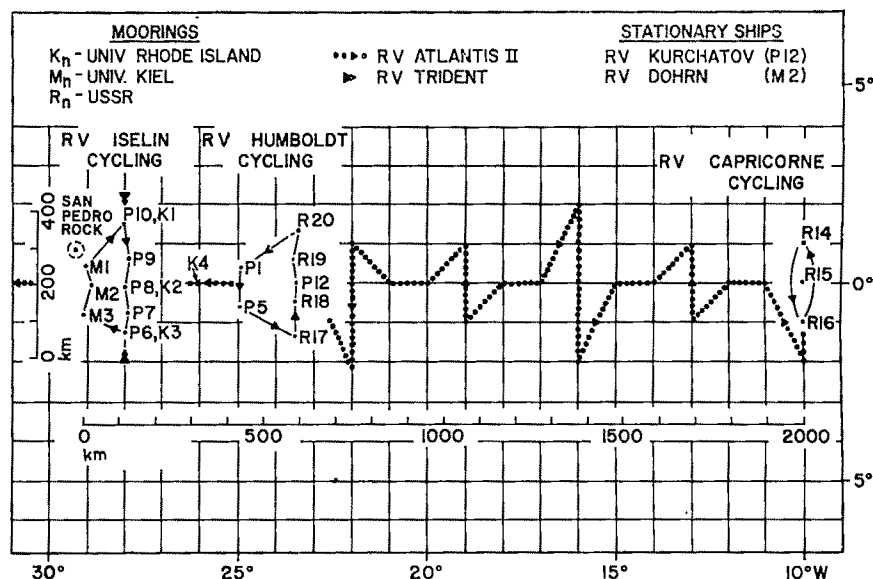


Fig. 2 Consecutive meridional sections of the east-west current component along 28°W observed by RV Iselin. Isotachs are in cm s^{-1} ; hatched areas denote westward flow.

profiling current meters (PCMs) and on their mode of operation see refs 4 and 5). The Humboldt used the SRS method⁶ to obtain complementary observations of current profiles.

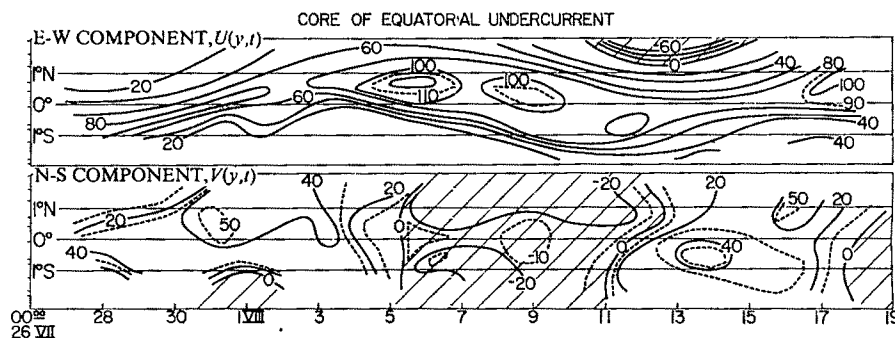
Meandering of equatorial currents

RV Iselin and RV Humboldt operated in cyclic modes along the western and the eastern trapezoids respectively (Fig. 1). This

procedure made possible the quasisynoptic observations that indicated meandering motions of the flow field as well as of the fields of temperature and salinity at all depths from the surface to well below the core of the Equatorial Undercurrent. Consecutive sections from July 26 to August 16 along 28°W show that the eastward-flowing core of the undercurrent meandered approximately between latitudes 0°50'S and 0°50'N (Fig. 2). Similar sections made by RV Humboldt along 23°30'W confirm that the meridional excursion of the core of the undercurrent occurred within ± 50 miles of the equator. Maximum core displacement took place over a period of 1 week at both, 28°W and 23°30'W. Thus, core displacements at both longitudes occurred on time scales much shorter than previously believed. The jet-like core of the undercurrent displays a pulsating behaviour: the volume transport in the core at 28°W, as obtained by integration within the 20 cm s^{-1} contour (Fig. 2), varied over a range from $4 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ on July 27 to $15 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ on August 5. This feature is also shown in the time-latitude sections in Figs. 3 and 4a, for example, by following the 60 cm s^{-1} isotach in the east-west component in Fig. 3. Pinching of the isotachs occurs around July 30 and around August 16; widening of the isotachs, accompanied by pronounced velocity maxima, occurs around August 7 at 28°W and about 3 d earlier at 23°30'W.

The westward surface flow was unusually strong, although intermittent, throughout the observational period. The westward core of the surface current showed meridional excursions similar to those observed at the level of the undercurrent (Fig. 5). The northerly component at position M2 (Fig. 6) indicates that fluctuations at the surface level at 28°W lag approximately 3–4 d behind changes at the undercurrent level. Such a vertical lag does not seem to occur at 23°30'W. Another phenomenon observed only at the western trapezoid was a

Fig. 3 Time-latitude sections of the flow components at the core level of the undercurrent based on all stations along 28°W and 29°W. Maximum eastward value from each profile was selected at depths varying between 60 and 90 m. Observations by RV Iselin.



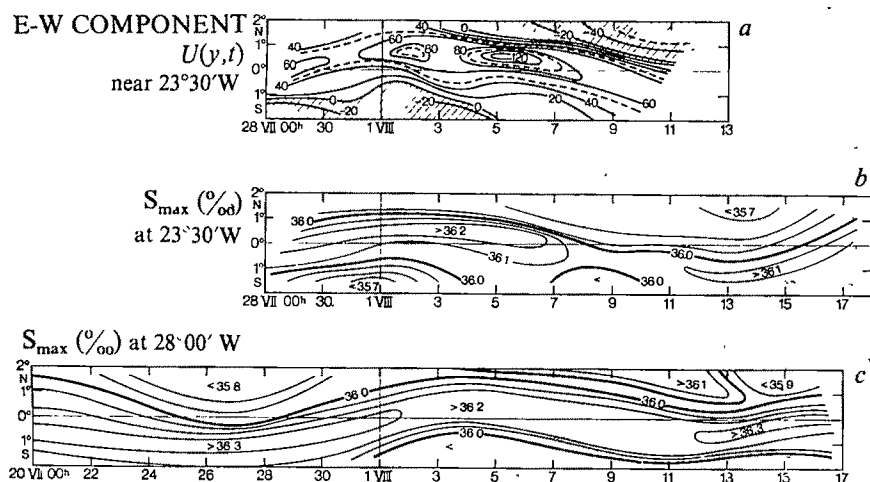


Fig. 4 Time-latitude sections of the east-west current component and of salinity. *a*, Observed by RV Humboldt at 23°30'W; otherwise corresponding to $U(y, t)$ -plot in Fig. 5; *b*, salinity maximum at 23°30'W observed by RV Humboldt; *c*, salinity maximum at 28°W combined from observations by RV Iselin and RV Trident.

surfacing of the undercurrent on August 10 at latitude 1°30'S when weak eastward flow occurred at the surface. By August 15, eastward flow at the surface was observed in the region from 1°30'S to 0°50'S. Remarkably, this happened in spite of prevailing winds from the south-east of about 7 m s⁻¹. This gives the impression that surfacing at 28°W is intrinsically linked to the meandering and wave processes of the equatorial currents.

Comparison of salinity sections (Fig. 4*b, c*) with sections of the eastern component Fig. 4*a* shows that the salinity maximum is a fairly reliable indicator of the meandering of the undercurrent, although the velocity core and the salinity maximum,

data to detect meandering and wave phenomena at other times and locations.

A meander period of about 16 ± 2 d is compatible with all observations presently on hand, including those before phase II of GATE. Corroborating observations were made by RV Kurchatov during phase I at a buoy station at 0°, 23°30'W. Bubnov *et al.*⁷ report results from a series of consecutive temperature casts made between June 28 and July 15, 1974. The temperature variability of the thermocline at the depth of the core of the undercurrent shows a pronounced 8-d periodicity. In conjunction with the dome-shaped temperature

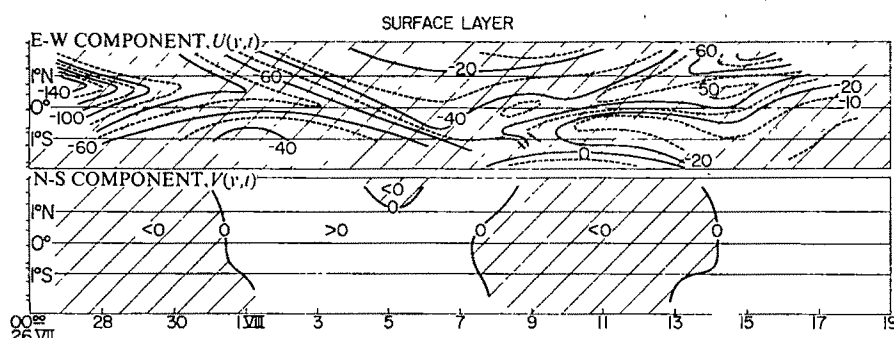


Fig. 5 Time-latitude sections of the flow components at the surface based on all stations along 28°W and 29°W. Values between 0 and 15 m were averaged. Hatched areas denote westward respectively southward flow. Observations by RV Iselin

in most cases, do not occur at the same depth. Usually the salinity maximum is found above the velocity core in the region of strongest vertical current shear. In spite of these differences in vertical structure, horizontal meandering of the undercurrent may be detected from salinity observations alone. For example, salinity sections made during ICITA⁶ at 8°W indicate meandering of the undercurrent with a half period of approximately 14 d, and with meridional excursions of the core to the north and to the south of the equator similar to the excursions observed further to the west during GATE. There is, however, some evidence from the observations by RV Atlantis II that such correlation does not always exist. If the correlation between the salinity field and the flow turns out to be sufficiently reliable, it may be possible to use historical salinity

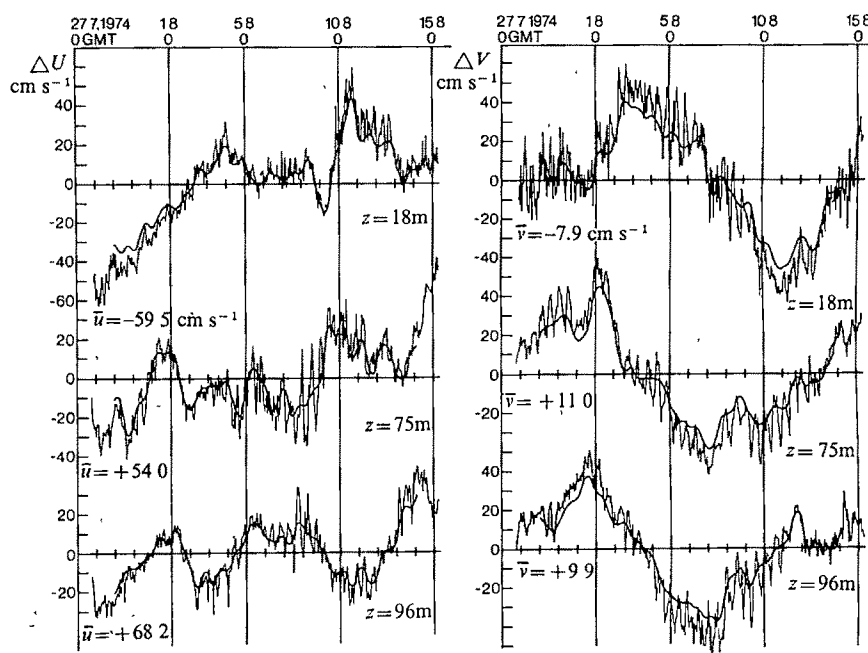
distribution at the same longitude between 1°30'N and 1°30'S. Bubnov *et al.* conclude that the undercurrent core meandered across the equator with a 16-d period.

Observations by RV Atlantis II at 28°W also fit this picture. Between July 15 and 17, the eastward core of the undercurrent at 28°W was located approximately 44 miles north of the Equator. The observations by RV Iselin (Fig. 3) show the undercurrent to be at its peak northerly excursion at about July 18–20, one-half period before the start of phase II. Furthermore, RV Atlantis II made 17 sections across the undercurrent from 10°W to 33°W before phase II. These observations establish that the undercurrent was frequently displaced off the Equator and also that meandering occurred within 0°50'N and 0°50'S.

Table 1 Eastward and westward propagating waves

Westward propagating waves			Eastward propagating waves		
$c_n = \frac{\omega}{k_n} = \frac{\Delta x}{\Delta T + nT}; n = 0, 1, 2, \dots$			$c_n = \frac{\Delta x}{nT - \Delta T}; n = 1, 2, \dots$		
Where $T = 16$ d, $\Delta T = 72$ h; $\Delta x = 500$ km					
n	$L(\text{km})$	$c(\text{m s}^{-1})$	n	$L(\text{km})$	$c(\text{m s}^{-1})$
0	2,660	1.93	1	615	0.45
1	432	0.31	2	271	0.20
2	231	0.17			

Fig. 6 Flow components in the upper layer observed at three fixed levels at $0^{\circ}29'W$ (Position M2) from a surface mooring deployed by RV Dohrn. Smooth line obtained from low pass filtering. Amplitude reduction for lower frequencies due to particular filter response.



Fixed level current meter records made from RV Dohrn during phase II confirm the previously mentioned meander time scales (Fig. 6). A particular feature may, however, be mentioned here since it has important ramifications when interpreting records from fixed level meters. Whereas the northward component shows basically the same long periods as those in Figs 3 and 5, the eastward component at the fixed levels at position M2, at $0^{\circ}29'W$ shows half that period ($\approx 8-9$ d). An apparent doubling with frequency 2ω will occur when a jet-like flow meanders back and forth with frequency ω across a fixed observational point. The representation in Fig 3-5 circumvents this problem.

Wave propagation

A study of the time-latitude distribution of the eastward component at the core level of the undercurrent at $28^{\circ}W$ (Fig. 3) shows a pattern that closely resembles the one observed at $23^{\circ}30'W$ (Fig. 4a). There are three specific features that are strikingly similar in both observations. (1) long period shifting of the undercurrent core across the Equator, (2) appearance of two cell-like maxima in the eastward component, and (3) development of westward flow north of the Equator towards the end of the observation period. The latter observation is confirmed by observations from RV Kurchatov at position P12 (ref. 8). The time-latitude distributions of the salinity maxima at both longitudes (Fig. 4b, c) are independent of the flow field, also show long period meandering of the salinity core and are in good agreement, even in some smaller scale features.

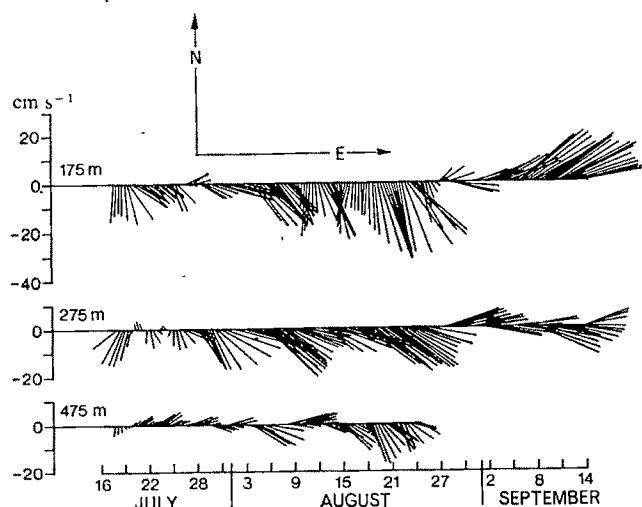
Both flow fields and salinity fields at the two longitudes which are separated by 500 km reveal that the pattern at $28^{\circ}W$ lags several days behind that at $23^{\circ}30'W$. Combining all information now available gives a lag estimate of $\Delta T \approx 72 \pm 7$ h. Using this information, simple geometric reasoning gives eastward or westward propagating waves according to Table 1. Thus, there are basically two alternatives: (1) very long westward moving waves, or (2) much shorter, westward or eastward moving waves. It turns out that the PCM observations from the eastern and western trapezoid (Fig. 1) support the assumption of a long westward propagating wave (S. A. Thorpe, personal communication) with $L = 2,600 \pm 390$ km and $c = 1.9 \pm 0.3$ m s $^{-1}$. Phase relationships between $28^{\circ}W$ longitude (positions P6 to P10) and $29^{\circ}W$ (positions M1 to M3) longitude were not sufficiently convincing to justify an interpretation in terms of short eastward (or westward) moving waves. A final determination of phase propagation in the various frequency bands is

expected to be forthcoming from current meter records of longer duration.

Observations made by RV Capricorne at $10^{\circ}W$ were carried out over a relatively short period, from July 30 until August 10; meridional station spacing was 60 miles, and resolution is therefore limited. A detailed comparison and phase alignment with the observations further to the west is therefore not possible. There is no doubt, however, that large scale meandering also occurs at this longitude. During phase II of GATE, the northward component of the flow was predominantly positive at the level of the undercurrent with values $v \approx 40 \pm 18$ cm s $^{-1}$, whereas the eastward component was $u = 85 \pm 19$ cm s $^{-1}$. This is in contrast to a sequence of 18 current profiles observed at 3-hourly intervals at $10^{\circ}W$ during August 1973 (ref. 5). At that time the mean value v was southward, $v \approx -42 \pm 12$ cm s $^{-1}$, at the level of the undercurrent, whereas $u \approx 55 \pm 15$ cm s $^{-1}$; that is, during both years, 1974 and 1973, the undercurrent veered between 25° and 35° northward or southward respectively from its course due east.

Concluding the observational part, we note that the 16-day wave or meander is the dominant feature in the surface layer

Fig. 7 Low pass filtered current vectors at three fixed levels below the core of the undercurrent at $0^{\circ}28'11'W$ (Position K2) from a subsurface mooring positioned by RV Trident.



and in the core level of the undercurrent. Additional observations by Meincke⁹, as well as those from a subsurface instrumented mooring positioned by RV Trident, clearly show that a 16-d wave is not a predominant feature at depths below 200 m. As shown in Fig. 7, the predominant oscillations are of both shorter and longer periodicities; of the order of 3–4 d and of the order of 1 month. These oscillations are coherent between 28°W and 26°W.

Probable cause of meanders

Cross-equatorial winds may displace¹¹ the core of the undercurrent from the Equator. An abrupt change in the winds could thus cause the undercurrent to meander across the Equator while it adjusts to the new wind field. It is, however, improbable that the meanders described (here) were generated in this manner because vessels on the equator on all longitudes from 10°W to 29°W found the winds to be remarkably steady throughout GATE.

A preliminary analysis (G. Philander, unpublished) of the stability properties of the equatorial currents reveals that the Equatorial Undercurrent was stable during GATE, but that the surface currents—the westward South Equatorial Current at and south of the equator, and the eastward North Equatorial Countercurrent to the north—had sufficient latitudinal shear to be inertially unstable. The curvature of the earth (β -effect) and equatorial divergence can both destabilise this current

system. The westward propagating unstable waves are predicted to have a wavelength of about 2,000 km, a period between two and three weeks, and an e-folding time of about one month. These numbers and the predicted phase lag between disturbances in the surface layers and those at greater depths are in reasonable agreement with the measurements described earlier.

If the surface currents are indeed unstable then the meander of the undercurrent at the equator is only part of a phenomenon with a much larger latitudinal scale. Furthermore, waves with a wavelength in excess of 2,000 km in an ocean basin which, at the equator, is barely 5,000 km wide, are likely to excite other modes of oscillation. Further analysis of the GATE data should provide answers and could potentially change our present view of the generating mechanism.

Note added in proof: Additional observations supplied by Dr V. A. Bubnov show that oscillations with comparable amplitude and period also occurred during phases I and III of GATE.

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External dose estimates for future inhabitants of Eniwetok Atoll

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Measurements of environmental γ radiation levels on Eniwetok Atoll were combined with pertinent population distributions and expected future life styles to assess the external doses that could be received by the Marshallese people on their return to the former US nuclear weapons test site in the Pacific.

WITH the prospect of habitation in the near future, a comprehensive survey of the total radiological environment of the Eniwetok Atoll was undertaken to provide a basis for determining whether or not the atoll can be safely rehhabited by the former Marshallese population¹. As an integral part of the total radiological survey plan, a study was made to assess the total external dose that the returning population might receive as a result of the radiological contamination distributed in the environs of the atoll. Eniwetok Atoll was one of the US nuclear weapons testing sites in the Pacific, and is situated in the northern part of Micronesia in the Central Pacific Ocean about 3,800 km south-west of Honolulu. The atoll consists of 40 islands on an elliptical coral reef surrounding a lagoon with major and minor axes dimensions of 37 and 27 km, respectively.

The islands, which were given male and female code names during the US occupancy, are shown in Fig. 1. The total land area is about 7 km² with the land height generally averaging 3–5 m above mean sea level. The islands vary in size from small sand bars of a few hundred square metres to heavily vegetated islands of about 1.5 km². The largest islands, and therefore those of most importance for future habitation, are Fred, Elmer and David in the southern part and Janet in the northern part of the atoll.

A total of 43 nuclear tests took place during the 1948–58 testing period. Most of the tests were conducted throughout the northern part of the atoll. Slightly over half of the nuclear devices were detonated over the lagoon and ocean areas whereas the remainder were detonated on several of the northern islands. As a consequence, the major radiological impact of the testing programme occurred throughout the northern islands with only minor radioactive contamination on the southern islands.

Since the external dose is almost entirely the result of γ -emitting radionuclides, with only minor contributions from α and β emitters, it was essential to obtain the best possible description of the geographical variability of the γ exposure rates in air on each island. The following is a brief description

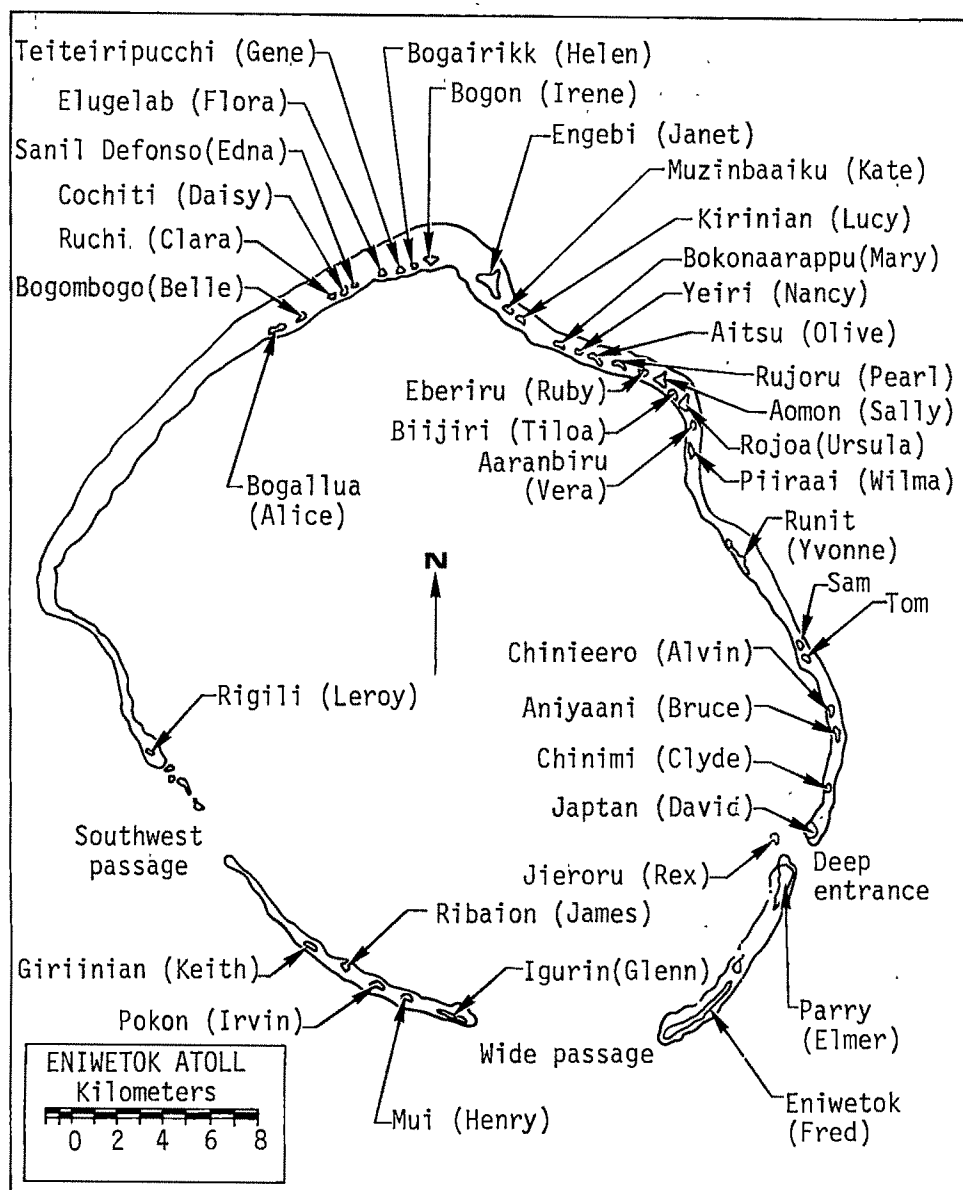


Fig. 1 Map of Eniwetok Atoll.

of the techniques used to measure these exposure rates and how the resulting data, in conjunction with pertinent population statistics and expected life style, enabled us to make realistic estimates of the external dose to future inhabitants.

Measurement techniques

Several independent techniques were used to measure these exposure rates since each technique has its own set of limitations (for example, nonlinear energy response, portability of equipment, and extent of geographical coverage). We used results from the following techniques to determine the total exposure rates at 1 m above the ground: (1) portable, hand-held NaI scintillation detector measurements at 1,000 locations distributed over all islands, (2) LiF and CaF_2 : Dy thermoluminescent (TLD) measurements at 100 locations on selected islands, and (3) a helicopter-borne array of 40 NaI crystal spectrometers flown in a grid pattern over each island. The third technique reveals that the primary contribution to the total exposure rates was caused by ^{137}Cs and ^{60}Co activities in the soil. Soil γ spectral measurements indicate that trace amounts of other γ emitters such as ^{125}Sb , ^{155}Eu , and ^{241}Am contribute at most 3–5% of the total exposure rate¹ and, therefore, were omitted in the dose evaluation.

Because of its energy linearity, excellent thermal stability and approximate air equivalency for a typical environmental

radiation field, we selected the LiF results as the reference to which measurements obtained by the other techniques could be compared. This comparison revealed that the LiF measurements are 17% less than those of CaF_2 . This is reasonable in view of the enhanced energy response of the CaF_2 at low energies. Most importantly, however, the aerial survey and the portable NaI detector results agree, on average, within 10% with the LiF measurements. This is within the accuracy of the measurements, and the agreement is considered excellent.

As expected, the highest exposure rates were measured on the northern islands. The highest average value of $115 \mu\text{r. h}^{-1}$ was recorded on Belle. The remaining islands typically have exposure rates of $10\text{--}80 \mu\text{r. h}^{-1}$. In general, the highest levels were found at centres of the island or in proximity to ground zero sites, and were usually related in a direct way to the vegetation density in the immediate area. The southern islands are characterised by uniformly distributed levels of less than $1 \mu\text{r. h}^{-1}$ (see ref. 1 for full details of data).

External dose determination

In addition to the γ -ray exposure rates, the expected living patterns of the future inhabitants must be considered to evaluate the external dose problem. Because of the uncertainties inherent in predicting future living patterns, several cases were

Table 1 Assumed geographical living patterns

Case description		Group	% Population	Village	% time spent in respective areas			
					Beach	Interior	Lagoon	Other islands
Ia	Village on Janet, visits to other northern* islands only	Infants	22	85	5	0	0	10
		Children	42	55	10	15	5	15
		Men	20	50	5	15	10	20
		Women	16	60	10	10	0	20
Ib	Village on Janet, visits to other northern* islands only	Infants	22	70	5	5	0	20
		Children	42	50	5	15	10	20
		Men	20	40	5	20	10	25
		Women	16	50	5	15	5	25
II	Village on Fred, Elmer or David, visits to northern* islands only (excluding Janet)	Infants				Same as case Ib		
		Children						
		Men						
		Women						
III	Village on Janet, visits to southern† islands only	Infants				Same as case Ib		
		Children						
		Men						
		Women						
IV	Village on Fred, Elmer or David, visits to southern† islands only	Infants				Same as case Ib		
		Children						
		Men						
		Women						

*Northern islands include Alice, Belle, Clara, Daisy, Irene, Janet, Kate, Lucy, Mary, Nancy, Olive, Pearl, Sally, Tilda, Ursula, Vera, Wilma.
†Southern islands include all islands from Tom to Leroy proceeding clockwise around the atoll.

chosen for analysis (Table 1). The selection was based on the most recent information available regarding present population figures, age distributions, and expected life styles¹. Furthermore, the cases were chosen in such a way as to bracket the most likely range of doses that could be received by any sizeable segment of the population (a total of 430 people). This will enable any other reasonable pattern to be inferred by proper interpolation of the results obtained for the cases shown in Table 1.

The cases are based on the assumption that some fraction of the population may choose to reside primarily on Janet (the largest island within the northern part of the atoll), with the remainder residing on Fred, Elmer, or David in the southern group of islands. Each case considered allows for visits to other islands. Case Ib differs from case Ia in that more time is allotted to temporary occupation of islands other than Janet at the expense of less time being spent in the Janet village area. These cases, or combinations thereof, are considered to represent the most likely living patterns.

Even though wide variations in γ -ray exposure rates were measured throughout the northern islands, it was necessary, for the purpose of calculating the dose, to derive the most reasonable values of the current mean exposure rates for each specific geographical area considered. These values are shown in Table 2. The mean exposure rates for specific areas of Janet were obtained by examination of the ^{137}Cs and ^{60}Co isoexposure-rate contour maps provided by the aerial survey. The village area was assumed to lie along the lagoon side of the island. The mean values given for all of the northern islands were obtained by weighting the mean exposure rates for each individual island with the area of each island. Since the minor contamination of the southern islands is relatively uniform, the mean ^{137}Cs and ^{60}Co exposure rates were chosen by inspection of the individual aerial-survey contour maps. The cosmic-ray contribution was estimated to be $3.3 \mu\text{r. h}^{-1}$ at this latitude (11°N) and the naturally occurring radionuclides in the soil and seawater were expected to contribute an additional $0.2 \mu\text{r. h}^{-1}$.

Integral 5, 10, 30 and 70-yr γ -ray doses for each age group were calculated for each case or living pattern described in Table 1. The results were then combined with the present population distribution, which is also shown in Table 1. Corrections were made for radioactive decay, but not for

possible weathering and subsequent deeper penetration of the radionuclides in the soil. The results of these calculations are given in Table 3 and are labelled 'unmodified.' Additional calculations were made to ascertain the effect of reasonable attempts to reduce the exposure rates on the atoll.

The first modification, labelled 'village gravelled' in Table 3, reflects the effect of covering the village areas with about 5 cm of uncontaminated coral gravel—a common practice throughout Micronesia. This action can be expected to reduce the γ -exposure rates in the village area by approximately a factor of 2. The second and third modifications are based on the assumption that clearing the islands for agricultural use and housing will result in some mixing of the topsoil. It seems that it would be practical during this period to also plough many of the more contaminated islands to a depth of 30 cm. Assuming that ploughing results in mixing rather than burying the topsoil, an average reduction in exposure rates of about a factor of 3 may be obtained. This is based on the present 3–5-cm relaxation lengths (the depth at which the activity is e^{-1} , or 37%, of the surface activity) for activity depth distribution in the uppermost soil layers of the more contaminated areas. This value, however, is highly variable from site to site. In Table 3, modification 2 indicates the effect of ploughing only Janet, whereas modification 3 reflects the additional effect of ploughing all the northern islands. Deeper ploughing

Table 2 Estimated mean exposure rates ($\mu\text{r. h}^{-1}$) used for dose calculations

Major geographical area	Source	Village	Interior	Beach
Janet	^{137}Cs	9.0	33	1.0
	^{60}Co	5.0	14	0.5
Fred, Elmer or David	^{137}Cs	0.2	0.2	0.2
	^{60}Co	0.1	0.1	0.1
Area-weighted mean exposure rates				
Northern islands (Alice–Wilma but excluding Janet)	^{137}Cs		14	
	^{60}Co		21	
Southern islands (Tom–Leroy)	^{137}Cs		0.2	
	^{60}Co		0.1	
All areas (including lagoon)	Cosmic and natural		3.5	

Table 3 Estimated integral external free-air γ doses (rad)

	Time interval (yr)			
	5	10	30	70
Ia/Unmodified	0.76	1.37	3.12	5.33
1. Village gravelled	(0.62)	(1.12)	(2.58)	(4.51)
2. + Janet ploughed	(0.41)	(0.75)	(1.77)	(3.27)
3. + Northern islands ploughed	(0.30)	(0.56)	(1.40)	(2.76)
Ib/Unmodified	0.83	1.49	3.35	5.65
1. Village gravelled	(0.71)	(1.28)	(2.89)	(4.96)
2. + Janet ploughed	(0.49)	(0.87)	(2.01)	(4.96)
3. + Northern islands ploughed	(0.33)	(0.61)	(1.50)	(2.90)
II/Unmodified	0.38	0.68	1.59	2.97
3. Northern islands ploughed	(0.22)	(0.41)	(1.08)	(2.26)
III/Unmodified	0.60	1.10	2.60	4.60
1. Village gravelled	(0.48)	(0.88)	(2.14)	(3.90)
2. + Janet ploughed	(0.25)	(0.48)	(1.26)	(2.56)
IV/Unmodified	0.14	0.28	0.83	1.92
Mean population dose (average of cases Ib-IV)				
Unmodified	0.49	0.89	2.09	3.79
1. Village gravelled	(0.43)	(0.78)	(1.86)	(3.44)
2. + Janet ploughed	(0.32)	(0.58)	(1.42)	(2.77)
3. + all northern islands ploughed	(0.24)	(0.45)	(1.17)	(2.41)
Sea level USA (80 mrad yr ⁻¹) typical	0.40	0.80	2.40	5.60

or turning over the soil rather than mixing it would, of course, result in even greater reductions in exposure rate. For example, mixing to a depth of 60 cm would reduce the exposure rates by an additional factor of 2, whereas covering the sources with approximately 30 cm of uncontaminated soil would essentially reduce the exposure rates to negligible values similar to those observed on the southern islands. Removing the top 15 cm of soil, which often contains about two-thirds of the activity, would result in a threefold reduction in the exposure rates. The advantages of ploughing or removing the topsoil should, however, be considered on a case-by-case

basis because of the highly variable distributions of activity with depth.

From Table 3 it can be seen that extensive modifications may not be required to reduce the dose levels to values comparable to typical US values². Keeping in mind that the selected cases represent approximations to the most likely living patterns, one observes that even for cases Ia and Ib, the unmodified 70-yr integral doses are comparable to the US values, although cases II and IV lead to considerably lower doses. The mean integrated doses shown in Table 3 were derived by averaging those for cases Ib, II, III and IV. This implies that half the returning population live on Janet and the other half live on Fred, Elmer or David and that trips to the northern or southern islands are equally likely for both groups. The unmodified mean population doses are all quite comparable with US values. At most, implementation of modifications 1 and 2 should be enough to assure mean population exposures well below the US levels.

Because of the low amount of natural radioactivity normally present in the coral atolls, the external dose levels calculated for cases I-III are still appreciably higher than corresponding levels found elsewhere in the Marshall Islands (essentially case IV). The results for cases II and IV indicate that restricting the permanent villages to 'clean' southern islands at least temporarily would result in lower exposures.

All of the doses discussed so far result from free-air γ -ray plus cosmic-ray exposures. The effect of shielding by structures or the body itself on gonadal or bone doses has been ignored. To convert from free-air dose (rads) to gonadal dose (rem), a body-shielding factor of 0.8 may be used³.

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¹ Eniwetok Radiological Survey, US Atomic Energy Commission Report NVO-140, Vol. I-III (Nevada Operations Office, Las Vegas, Nevada, 1973).

² Beck, H. L., Lowder, W. J., Bennett, B. G., and Condon, W. J., *Further Studies of External Environmental Radiation*, USAEC, Health and Safety Laboratory Report HASL-170, Table IV (1966).

³ Report of the United Nations Scientific Committee on the Effects of Ionizing Radiation. Levels and Effects, Vol. I, Levels, Annex A, 38 (1972).

Demonstration and origin of six tertiary base pair resonances in the NMR spectrum of *E. coli* tRNA^{Val}₁

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The 360-MHz NMR spectrum of E. coli tRNA^{Val}₁ reveals 26 resonances, six of which are derived from tertiary base pairs. The origin of these tertiary resonances and their approximate positions in the spectrum are discussed in relation to the crystal structure of tRNA.

EACH Watson-Crick base pair generates a single low field nuclear magnetic resonance (NMR) derived from the ring NH hydrogen bond¹. Kearns *et al.*^{2,3} showed that the ring NH hydrogen bonds from base pairs in tRNA in H₂O solutions, although solvent-exchangeable, possessed

adequately long helix lifetimes to generate discrete resonances with chemical shifts in the -11 p.p.m. to -15 p.p.m. region. High resolution NMR studies of several class I tRNA species (20±1 cloverleaf base pairs) have been carried out; the spectra were interpreted to contain 20±1 low field resonances from secondary base pairs, that is it was claimed or assumed that no extra resonances from tertiary base pairs were present²⁻¹⁴.

We recently showed that, of all the class I tRNA species so far studied, *Escherichia coli* tRNA^{Val}₁ exhibited the best-resolved low field NMR spectrum; integration of the 270-MHz spectrum of this tRNA on the assumption that several resolved peaks contained a single proton, indicated

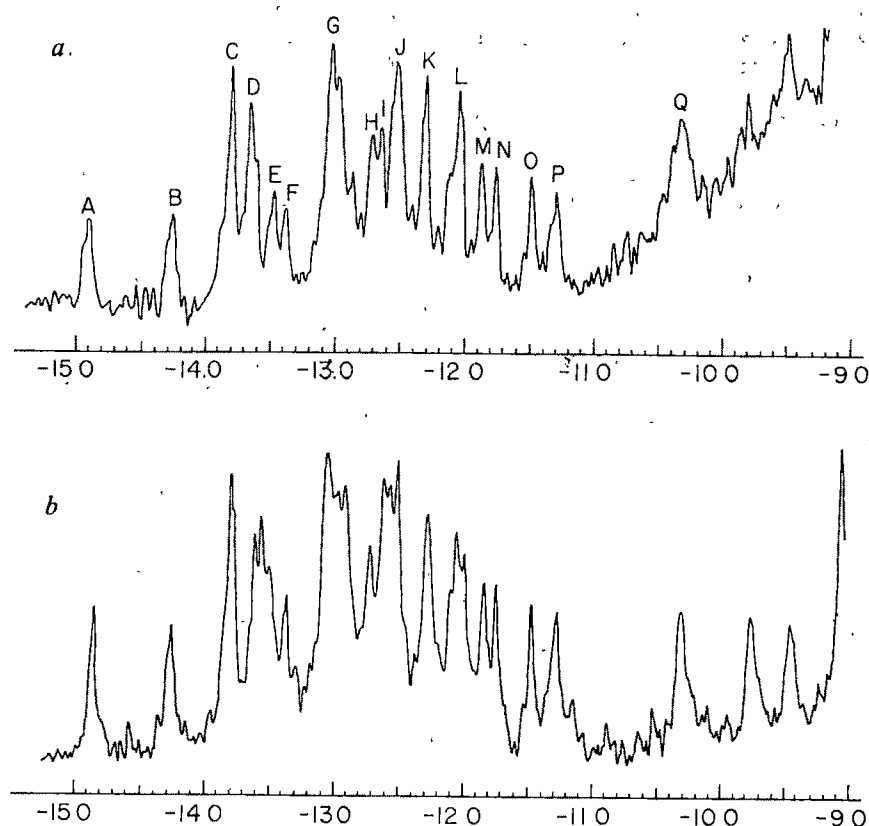


Fig. 1 360-MHz spectrum of *E. coli* tRNA₁^{Val} in the presence (a) and absence (b) of magnesium. The solvent for the upper spectrum was 10 mM Na cacodylate, 15 mM MgCl₂, 0.1 M NaCl, 1 mM EDTA, pH 7.0. The solvent for the lower spectrum was 10 mM Na cacodylate, 10 mM EDTA, pH 7.0. In both samples the tRNA concentration was approximately 25 mg ml⁻¹ (about 1 mM) and the spectra were signal averaged for several hours to improve signal-to-noise ratio.

that, contrary to previous interpretations, the spectrum contained several extra resonances derived from tertiary base pairs¹⁵. We have now reinvestigated *E. coli* tRNA₁^{Val} at 360 MHz; the improved resolution reveals six tertiary base pairs in the solution structure of this tRNA. The demonstration of 26 ± 1 base pairs is independently corroborated by calibrating the intensity of the low field spectrum with respect to the intensity of the methyl resonances of the three methylated bases in the molecule.

Internal calibration of 360-MHz spectrum

E. coli tRNA₁^{Val} was purified to 98% homogeneity by standard chromatographic procedures. Figure 1 shows the 360-MHz spectrum at 35 °C; in the presence of Mg²⁺ (Mg²⁺-tRNA ratio of 15) and in the absence of magnesium. In the magnesium-containing spectrum there are 16 resolved peaks between -11 p.p.m. and -15 p.p.m. In our previous spectra at 270 MHz we were able to integrate

with respect to the single proton peaks at -14.9 p.p.m. and -14.3 p.p.m. and establish that the total intensity corresponded to 26 ± 3 protons. Now, with the superior resolution at 360 MHz, we can integrate most peaks individually. Peaks A, B, E, F, M, N, O, P have the same intensity which we assume to correspond to one proton. Based on this assumption peaks C, D, K and peak H+I correspond (with an error of less than 10%) to two protons. Peak J contains three protons and peak G contains five protons, peak L corresponds to an intensity significantly greater than two protons. The experimental peak intensities and their nearest integral values are listed in Table 1. Thus integration of resolved individual peaks enables us to determine the overall intensity with much greater accuracy and leads to a value of 26 ± 1 protons. Since there are only 20 secondary base pairs in this tRNA (Fig. 3) this establishes that there are at least six tertiary base pairs involving ring N · · · H bonds in the solution structure.

Table 1 Integrated intensities of individual peaks in the 360 MHz spectrum of *E. coli* tRNA₁^{Val}

Peak	Position (p.p.m.)	Intensity	Integral value
A	-14.9	0.8	1
B	-14.3	1.0	1
C	-13.8	2.0	2
D	-13.7	2.1	2
E, F	-13.5, -13.4	1.9	2
G	-13.0	5.0	5
H, I	-12.7, -12.6	2.1	2
J	-12.5	3.0	3
K	-12.3	1.9	2
L	-12.1	2.4	2-3
M	-11.9	1.0	1
N	-11.8	0.9	1
O	-11.5	0.9	1
P	-11.3	0.9	1
Total			26 ± 1

Independent calibration based on methyl resonance intensity

The validity of the assumption that the resolved small peaks in Fig. 1 contain one proton can be justified on the grounds that, if they contained two protons the spectrum would indicate 54 base pairs which is impossible with only 76 nucleotides. Since the conclusion that six resonances from tertiary base pairs were present in the spectrum is, however, contrary to all previous interpretations of class I tRNA spectra, we felt it was important to calibrate independently the intensity of a single proton.

A sample of 11 mg of *E. coli* tRNA₁^{Val} was dissolved in 2.1 ml of water and two samples of 1.00 ml were lyophilised in separate small tubes. The first sample was dissolved in 0.20 ml of D₂O and the high field spectrum in the methyl region was taken; the second sample was dissolved in 0.20 ml of the normal H₂O buffer and the low field spectrum taken as usual. Both spectra were accumulated

for the same number of sweeps at the same r.f. power. Figure 2 shows the two spectra. There are three resolved peaks of equal intensity at -2.8 p.p.m., -2.6 p.p.m. and -1.1 p.p.m. in the region expected for methyl resonances. Figure 3 shows the cloverleaf structure of *E. coli* tRNA_I^{Val} which contains three methylated bases, namely m⁶A37, m⁷G46 and rT54. Thus the three methyl peaks in the high field spectrum define the intensity of three protons at the same spectrometer settings used in the low field spectrum. Peaks A, B, M, N, O, P all have an intensity between 30% and 35% of the methyl intensity, thus establishing unequivocally that they are in fact single protons. Furthermore, the overall intensity of the low field spectrum is 8.5 times the methyl resonance intensity, again corroborating the presence of 26 ± 1 base pairs.

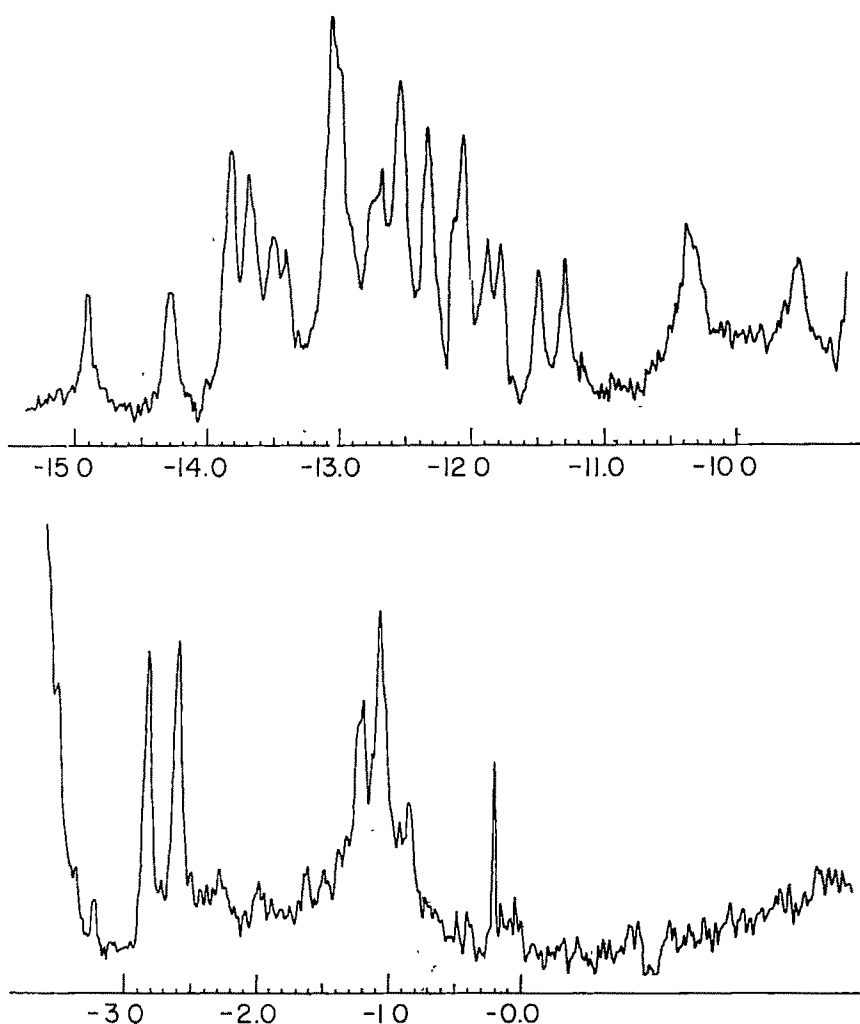
Previous assignments of methyl resonances in tRNA by Kan *et al.*¹⁶ enable us to assign the peak at -1.1 p.p.m. to rT54. The peaks at -2.8 p.p.m. and -2.6 p.p.m. must be m⁷G46 and m⁶A37 and, based on the data of Kan *et al.*¹⁶, the -2.8 p.p.m. peak can be tentatively assigned to m⁷G46. The peak at -1.2 p.p.m. has an intensity of approximately 2 protons and is probably the methylene protons of the oxyacetic side chain of residue V34 in the wobble position of the anticodon triplet. The peak at -0.2 p.p.m. is a solvent contaminant, but there is still some rather diffuse intensity between -0.7 p.p.m. and -2.5 p.p.m. which may be derived from hU17.

Base pairs contributing tertiary resonances

The crystal structure of yeast tRNA^{Pho} has been determined at 3 Å resolution^{17,18}. The fact that almost all

positions involved in tertiary bonding in yeast tRNA^{Pho} are occupied by the same nucleotide in *E. coli* tRNA_I^{Val}, together with more detailed arguments on the general structure of class I tRNAs^{19,20}, prompted us to consider the origin of the tertiary base pair resonances on the basis of the crystallographic data. The five known tertiary interactions involving ring NH hydrogen bonds are shown in Fig. 4. Four of these involve ring NH...ring N bonds and one (G15-C48) involves a ring NH which bonds to an exocyclic carbonyl oxygen; hydrogen bonding to carbonyl oxygens is less deshielding and would be expected to generate a ring NH resonance towards the high field end of the -11 to -15 p.p.m. region¹. There are nine tertiary interactions seen in the crystal structure^{17,18}; however, three of them (A9 to AU12; A21 to A14; G26 to A44 or A26 to G44 in tRNA_I^{Val}); involve exocyclic amino hydrogen bonds instead of ring NH bonds and thus would not be expected to contribute to the -11 to -15 p.p.m. region of the spectrum¹. The last tertiary pair is G18-ψ55. The precise bonding in this interaction has not been determined unambiguously; however, recent data (A. Rich, personal communication) indicate that it involves a ring NH proton which probably bonds to a ribose oxygen. Such bonding would lead to a tertiary resonance which would also be expected to be towards the high field end of the -11 to -15 p.p.m. region of the spectrum. The crystallographic data showing five definite and one probable tertiary interaction involving ring NH bonds, and the data presented here showing six tertiary ring NH resonances, are in excellent agreement. Our data are completely consistent with the crystal structure being the actual structure in solution.

Fig. 2 Low field hydrogen bond and high methyl spectra of *E. coli* tRNA_I^{Val} samples at identical concentrations. Duplicate samples (26.2 mg ml^{-1}) were prepared as described in the text in D₂O containing 15 mM MgCl₂, 0.1 M NaCl (lower spectrum) and in 10 mM cacodylate, 15 mM MgCl₂, 0.1 M NaCl, 1 mM EDTA, pH 7.0, H₂O buffer (upper spectrum). Both spectra were accumulated at the same r.f. power for 1,500 sweeps of 16 s each at a temperature of 38 °C.



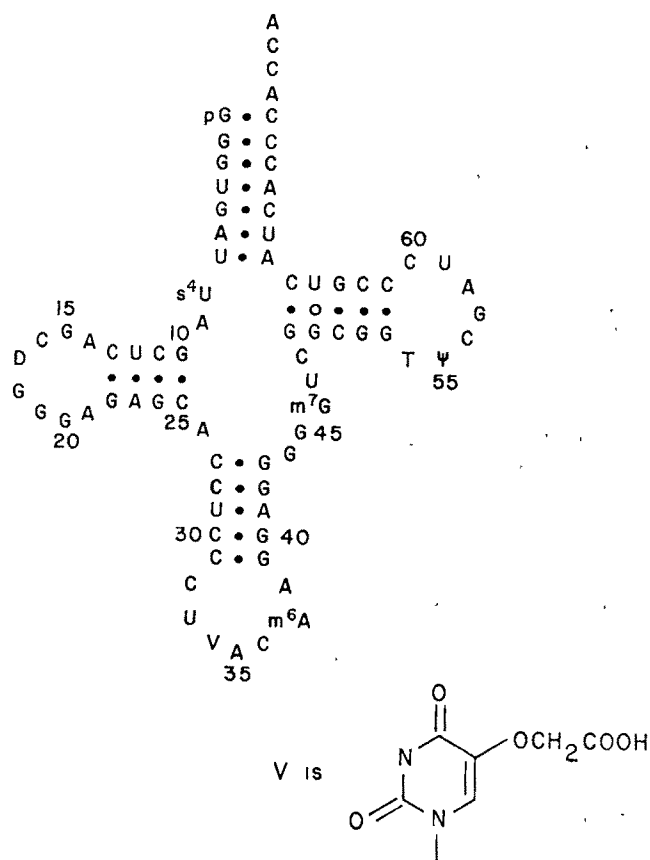


Fig. 3 The cloverleaf structure of *E. coli* tRNA₁^{Val} as reported by Yaniv and Barrell²³ and by Kimura *et al.*²⁴.

Expected chemical shifts for tertiary resonances

The environment of the various tertiary base pairs in the crystal structure, together with the approximate ring current shift contributions from neighbouring bases determined previously³, can now be used to estimate the approximate position of these tertiary resonances. The s⁴U8-A14 tertiary interaction has been assigned to peak A at -14.9 p.p.m. by chemical modification with cyanogen bromide¹³, additional modifications of this unique sulphur in *E. coli* tRNA₁^{Val} and *E. coli* tRNA₁^{Arg} by Wong *et al.*²¹ agree with this assignment. The reason for the extreme low field position of this tertiary resonance is the inherently

greater deshielding of the ring NH of s⁴U compared with U.

The T54-A58 interaction involves a ring NH...ring N bond. It is stacked with G18-ψ55 on one side and with G53-C61 on the other side; hence this resonance will be only moderately upfield shifted by neighbouring ring current effects. Since AT pairs have similar resonance positions to AU pairs²², we would expect the T54-A58 resonance to be somewhere in the -13.5 to -14 p.p.m. region, that is peaks C, D, E or F (peak B is in the position expected for the secondary AU6 pair).

The G19-C56 interaction is a standard Watson-Crick pair with a ring NH...ring N bond. This tertiary pair is interesting in that it constitutes one extremity of the molecule with no residue on one side and G57 stacked on the other side. Thus, this resonance would be only moderately shifted from -13.6 p.p.m. to somewhere in the -12.7 to -13.3 p.p.m. range, that is peak G, H or I.

The m⁷G46 to G22 tertiary interaction is a ring NH bond from m⁷G46 to the ring N of G22. Its environment involves stacking with A21, A14, A9 and A23¹⁹. Since adenine is the most potent ring current shift base³, this resonance would suffer an extremely large upfield shift—perhaps 2 p.p.m. or more. Thus the 46-22 tertiary resonance would be expected in the -11 to -12 p.p.m. region (peak M, N, O or P).

The 15-48 and 18-55 tertiary interactions involve ring NH protons which do not bond to ring nitrogens but instead bond to oxygen atoms. Such bonding has been shown to be less deshielding¹ and in addition these bonds would suffer an average upfield ring current shift from their stacking neighbours¹⁹. Thus the 15-48 and 18-55 resonances would be expected at the high field end of the spectrum between -11 and -12 p.p.m., that is peak M, N, O or P.

To summarise, the 8-14 interaction is at -14.9 p.p.m. and the 54-58 and 19-56 interactions are probably around -13.7 p.p.m. and around -13.0 p.p.m. respectively. The crystallographic data also lead us to predict that the three remaining tertiary resonances—15-48, 18-55 and 46-22—will all be in the -11 to -12 p.p.m. region, the spectrum reveals four protons in this region and the secondary pair G5-C68 is predicted to be at -11.6 p.p.m.

Tertiary structure in absence of magnesium

The low spectrum in Fig. 1 is that of a sample dialysed for 48 h against three changes (61 each) of double distilled water, lyophilised, and then dissolved in 10 mM sodium cacodylate, 10 mM sodium-EDTA, pH 7.0, at a concentration of 1 mM tRNA. The sodium ion concentration in the

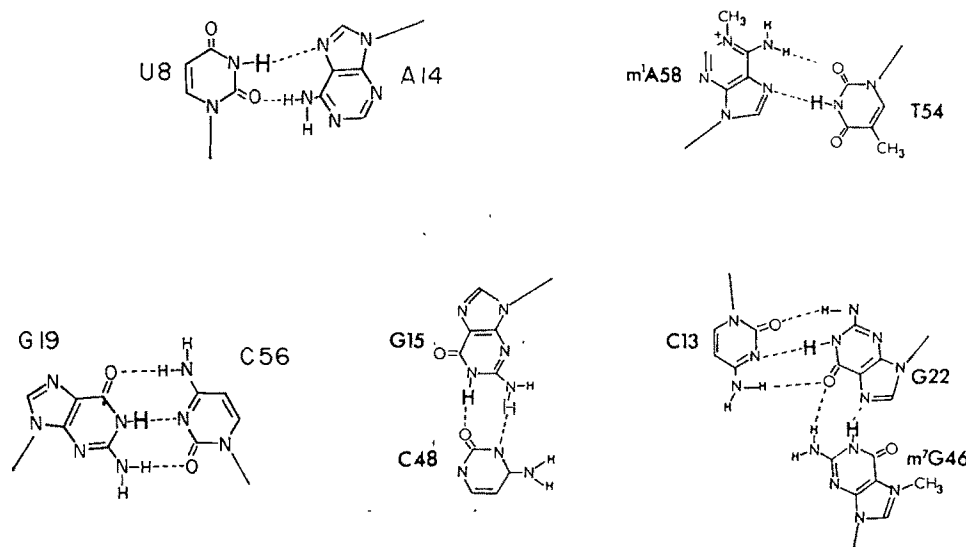


Fig. 4 Tertiary hydrogen bonding interactions seen in the crystal structure of yeast tRNA^{Phe} determined by Kim *et al.*^{17,19} and by Robertus *et al.*¹⁸ and Klug *et al.*²⁰. Only interactions involving ring NH hydrogen bonds are shown and these are denoted in bold face.

added solvent is 40 ± 5 mM. The lyophilised tRNA is the sodium salt and, at 1 mM, would generate another 75 ± 5 mM sodium ion. This excess EDTA-no magnesium solvent was chosen in the hope of destabilising tRNA tertiary structure. The 35 °C (and also the 45 °C) spectrum revealed, however, that all 26 resonances are still present. There are only two resonances which shift slightly in the non-magnesium structure, peak E (-13.5 p.p.m.) shifts downfield slightly into peak D, and peak I (-12.7 p.p.m.) shifts upfield slightly into peak J. Although it is difficult to interpret unambiguously such a subtle change, our current hypothesis is that the stacking of the acceptor helix on the rT helix (base pair 7 on base pair 49) is changed from the imperfect 17° stack¹⁷ to a more colinear stack. Concomitant with this change is a noticeable sharpening of the resonance at -10.4 p.p.m. We have assigned this resonance to the secondary G50-U64 pair¹⁵ which is presumably more protected from solvent exchange in the new structure.

The major conclusion to be drawn from these two spectra, however, is that all the tertiary interactions are formed even in the absence of magnesium; in fact the lack of spectral changes in all the other resonances suggests that the non-magnesium structure is remarkably similar to the magnesium-stabilised structure. Stabilisation of the structure by traces of residual bound magnesium can be discounted since heating the 1 mM tRNA sample in 10 mM EDTA to 65 °C for 1 h followed by subsequent cooling to 35 °C, resulted in a spectrum identical to the lower spectrum in Fig. 1.

Relation to earlier tRNA NMR data

We have now shown that *E. coli* tRNA_I^{Val}, with 20 secondary base pairs, contains six tertiary base pair resonances in its low field NMR spectrum; do the other class I tRNAs studied previously really have no tertiary resonances as claimed, or have their spectra been misinterpreted? The spectrum of yeast tRNA^{Phe} has been studied in greatest detail and has assumed the role of a 'reference spectrum'^{2-6,8-12}. The integration of low field spectra has been complicated by the lack of suitable internal standards with such large chemical shifts. Using Met-cyanomyoglobin as an external standard Wong *et al.*⁴, after normalising the two spectra to the same molar concentrations and the same number of accumulated sweeps, arrived at an intensity of 18.6 protons in the total spectrum. A composite peak at -13.7 p.p.m. was found to contain slightly more than three protons; this peak was normalised to 3.00 protons and subsequently became an 'internal stan-

dard'. A more recent revision by Kearns *et al.*¹⁰ reported a value of 19.7 protons for the total intensity of the yeast tRNA^{Phe} spectrum, but again made the assumption that the -13.7 p.p.m. peak contained three protons. We have re-examined the low field spectrum of yeast tRNA^{Phe} at 360 MHz (manuscript in preparation). At this resolution the peak at -13.7 p.p.m. resolves into three peaks of intensity 1:2:1, that is it contains four protons. Since this peak has previously been assumed to contain three protons and was used as an internal standard, it follows that the true intensity should be 1.33 times higher than reported. We note that 1.33 times 19.7 is in fact very close to 26. Independent integration of the yeast tRNA^{Phe} 360-MHz spectrum, which resolves into 14 peaks instead of the nine peaks in the 300-MHz spectrum, leads to a value of 26 ± 1 protons. Thus, we believe that all class I tRNAs probably contain approximately six tertiary resonances in their low field spectra, and previous NMR analyses have been erroneously interpreted.

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letters to nature

X-ray outburst from the direction of the galactic centre

WHEN the Uhuru satellite examined the X-ray emission from the region of the galactic centre in 1971 an extended region of emission centred on the radio source Sgr A and about 2° in extent was observed¹. We report here details of a transient point X-ray source, A1742-28, which has been observed at the centre of this region by the Ariel V rotation modulation collimator (RMC) experiment. Its rise to a peak and subsequent decline by a factor of two over 12 d were monitored. The

position was found to be $\alpha = 17^{\text{h}} 42^{\text{m}} 26.0 \pm 4.8^{\text{s}}$, $\delta = -28^{\circ} 59.8 \pm 1.2'$ (1950.0 coordinates, 90% confidence limits).

Figure 1 shows the intensity variation of the new source. Observations were made primarily in the photon energy range 3.0 to 7.5 keV although some data were obtained in the range 4.6-12 keV. Each plotted point represents observations spread over a period of one orbit (101 min). The RMC experiment is primarily designed to measure positions of point sources and has a grid pitch to separation ratio of 112, corresponding to an image response with full width at half maximum (FWHM) of

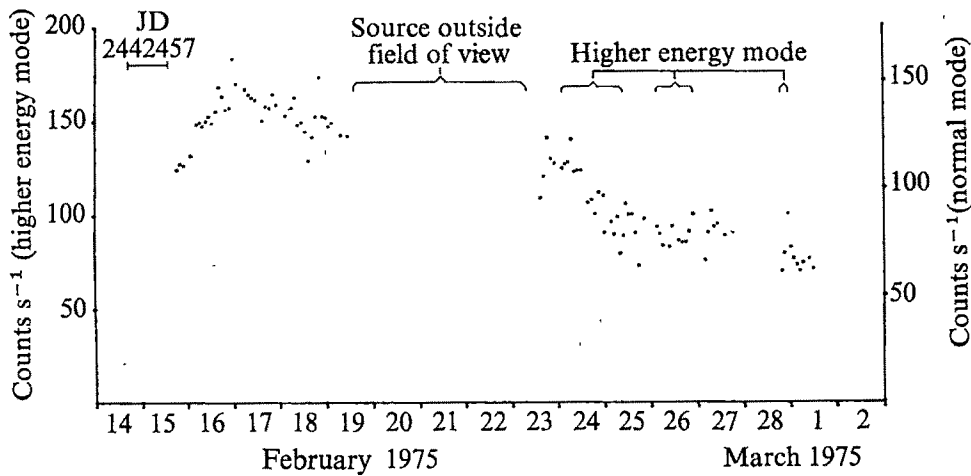


Fig. 1 The X-ray light curve of the transient A1742-28.

15' for a point source². Thus, we expect little or no response to extended sources larger than this and the intensities plotted correspond entirely to the new point source. The method of analysis occasionally leads to relatively large errors in intensity in regions as complex as that around the galactic centre where several other sources are always within the 17° FWHM field of view of the experiment. Thus, we do not consider the occasional large deviations from a smooth curve as necessarily significant.

In Fig. 1 the data points in the higher energy mode have been approximately normalised to bring them on to the same curve as the rest of the observations. This normalisation factor, together with the crude three-channel pulse height information available within each energy band have been used to deduce an approximate spectrum. Our findings are in reasonable agreement with those of J. C. Ives (personal communication) who reports a power law spectrum with a photon number index of about -3 and N_H approximately $2 \times 10^{23} \text{ cm}^{-2}$.

For comparison with counting rates quoted in the Uhuru (3U) catalog our figures should be multiplied by about 13. At the peak of about 170 counts s^{-1} the observed flux is about $4 \times 10^{-8} \text{ erg s}^{-1} \text{ cm}^{-2}$ in the energy range 3–10 keV.

Observations by another experiment on the same satellite indicate that the source was not present at comparable intensity

96 d before our observations commenced (K. Pounds, personal communication). No data are available for the period after March 1.

We have determined the coordinates of the new source using nearby X-ray sources of accurately known positions as standards. Lunar occultation positions for GX5-1 and GX3+1 are available^{3,4} and the positions of GX349+2 and GX9+1 are well established^{5,6} while 3U1700-37 has an optical counterpart. All of these sources were observed more or less regularly at the same time as the new source thus enabling checks to be made on the self-consistency of the results. From these checks we conclude that there are as yet unresolved systematic variations within our positional determinations. The size of the error box shown surrounding our best fit position in Fig. 2 results chiefly from the systematic effects, the random errors being only of the order of $10''$.

It is clear from Fig. 2 that the source lies very close to the direction of the radio source Sgr A and the associated infrared emission region. We have also observed a number of other broadly similar transient X-ray sources^{7,8}. The probability of a chance alignment of such an event with this particular direction, although difficult to estimate in retrospect, is certainly low. Given that an event occurred within the field of view of the experiment during the observations of that region, the proba-

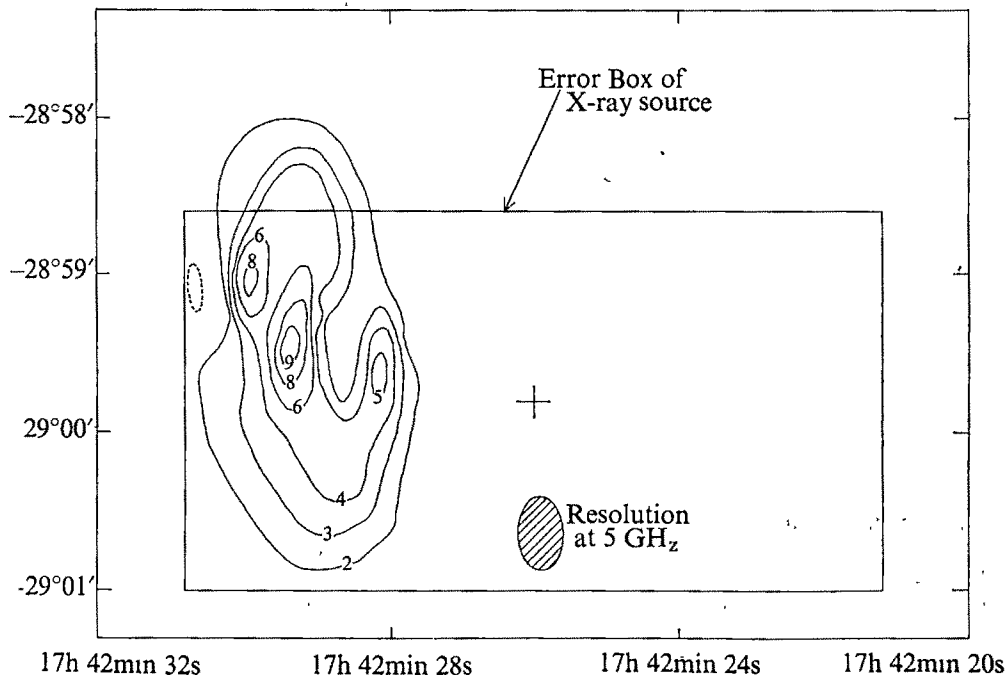


Fig. 2 The position and error box for the new source compared with a 5-GHz radio map of Sgr A west¹⁰.

bility of a chance alignment within $2'$ of Sgr A itself is $\lesssim 10^{-4}$. The probability that an event anywhere in the sky should be so aligned is of course lower than this. The probability of a similar alignment with any one of the 10 or so X-ray sources and other important features within this region is only an order of magnitude higher. It might be argued that the locally high stellar density close to Sgr A makes the occurrence of a transient similar to those observed in other regions very much higher. If the event occurred even close to Sgr A the distance must be ~ 10 kpc and this implies a peak flux of $5 \times 10^{38} \text{ erg s}^{-1}$ (3–10 kV), which is at the upper limit of the range of values usually encountered in galactic X-ray sources, perhaps suggesting again that this is a different type of phenomenon.

It is thus likely that the X-ray source is directly associated with one of the radio or infrared sources that lie within Sgr A. The fact that the rise and turnover of the light curve occurred on a time scale of the order of 2 d probably implies an emission region less than 0.002 pc in diameter. We note that long baseline interferometer studies have shown the presence of a source of less than $0.1''$ diameter (equivalent to 0.005 pc) in Sgr A west⁹, with a brightness temperature in excess of 10^9 – 10^7 K. A confirmation of this suggested association would require a change in radio or infrared emission from this region.

We thank J. C. Ives and K. A. Pounds for communicating their results before publication. We understand that a radio detection has been made by Jodrell Bank (personal communication).

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Drifting subpulses and pulsar slowdown rates

MANY models^{1–3} have been proposed to explain the observed radiation from pulsars, but none has yet explained satisfactorily all the features of pulsar emission. Studies of the relationships between pulsar properties may help in the understanding of the nature and evolution of pulsars and we present here evidence for a close relationship between the direction of drifting of subpulses from a pulsar and its position in a P – \dot{P} diagram—the plot of the derivative of a pulsar's period, \dot{P} , against period, P .

In such a diagram (Fig. 1), derived from the data of Lyne, Ritchings and Smith⁴, pulsars in the top, left hand corner of the diagram are thought to be young. They evolve downwards and towards the right hand edge of the triangular distribution of points where the pulse emission mechanism breaks down and the pulsars 'die', for reasons predicted by the various models. Interestingly, those pulsars with complex pulse profiles or

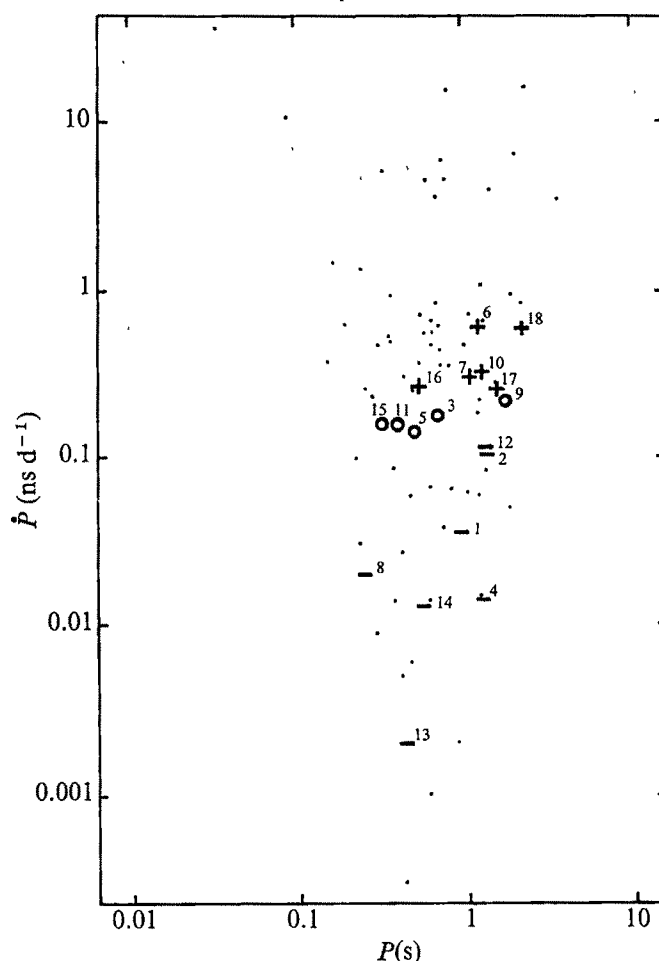


Fig. 1 A plot of the period derivative, \dot{P} , against the period, P , for 84 pulsars⁴. +, Subpulses drifting later, —, drifting earlier; o, drifting in both directions. Numbers correspond to those given in Table 1.

drifting subpulses, which may be a sign of pulsar 'old age'⁵, lie close to that edge.

Single pulse observations of several pulsars have been made using the Mark 1A radio telescope at Jodrell Bank, and sequences of pulses showing subpulse drift have been recorded for the pulsars PSR1112+50, PSR1642–03, PSR1944+17 and PSR2319+60. The subpulses received from PSR2319+60 drift later, that is, they drift from the leading edge of the integrated pulse profile towards the trailing edge, whereas those from PSR1944+17 move in the opposite direction. PSR1112+50 and PSR1642–03 display sequences in which the subpulses drift in either direction. These 4 pulsars, together with 14 others whose subpulse drift has been discussed in the literature are listed in Table 1. Subpulse drift from PSR1237+25 has been reported by Backer^{6,13}, but in view of the comments of Taylor *et al.*⁷, it has not been included in Table 1. Each pulsar in Table 1 is also indicated in Fig. 1, and the direction of the subpulse drift is shown.

From Fig. 1 there seems to be a trend indicating that subpulses from pulsars of high \dot{P} drift later and that those from low \dot{P} pulsars drift earlier. The change in drift direction occurs at $\dot{P} \sim 0.2 \text{ ns d}^{-1}$. The pulsars showing drifting subpulses have been selected by observers principally on the basis of flux densities, since a good signal-to-noise ratio is required for the detection of any drift. It is not evident, however, how that or any other selection effect could give rise to the trend, although we cannot rule out completely the possibility that it is produced by the insufficient sampling of pulsars which have a random distribution of drift directions.

It is widely believed that subpulses are associated with

discrete sources of emission and that subpulse drift is caused by the sources moving asynchronously with the pulsar rotation rate, although it is not generally clear what determines their motion. The direction of motion of the sources of emission has been defined, however, only in the model of Ruderman and Sutherland³ and then the observed direction of drift will depend on the observer's line of sight relative to the pulsar magnetic pole and the rotation axis. It would be remarkable if a chance set of orientations has produced the effect shown in Fig. 1, and subsequent studies of the subpulse drift in more pulsars may exclude this possibility.

It seems that some form of dragging on the particle bunches

lead to a better understanding of the physics of pulsars.

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Table 1 Pulsars with drifting subpulses

Pulsar	Pulsar number in Fig. 1	Drift direction	Ref.
0031-07	1	—	7,9
0301+19	2	—	9,11
0329+54	3	0	12
0809+74	4	—	7,9
0823+26	5	0	8
0834+06	6	+	7
0943+40	7	+	9,10
0950+08	8	—	8
1112+50	9	0	This paper
1133+16	10	+	7
1642-03	11	0	This paper, 6
1919+21	12	—	7,8
1944+17	13	—	This paper, 9
2016+28	14	—	7
2020+28	15	0	9,11
2021+51	16	+	7
2303+30	17	+	8,10
2319+60	18	+	This paper

+, Subpulses drifting later; —, subpulses drifting earlier; 0, subpulses which drift in both directions.

responsible for the subpulse radiation may be associated with the pulsar braking. This may not be significant for those pulsars with low values of \dot{P} (weak braking) and subpulses would drift earlier, but for high values of \dot{P} the dragging could be large and the particles may be 'forced' to move with different speeds, possibly in the opposite direction, thus producing the variation of subpulse drift direction with \dot{P} seen in Fig. 1. The exact nature of the association between the braking and dragging is not clear, but may be concerned with the magnetic dipole moment of the pulsar.

The dipole moment, M , is generally thought to be related to the rate of slowing down of the pulsar, according to $\dot{P} \propto M^2 P^{2-n}$, where n is the pulsar braking index which has experimental and theoretical values in the range 2.33–3 (see refs 1–4). In a plot such as Fig. 1, the evolutionary paths of pulsars would then be straight lines with slopes of between -0.33 and -1 and the wide range of \dot{P} would result from a spread in initial values of the dipole moment. If that is the case it would seem that subpulses drift later in pulsars with large dipole moments and in the opposite direction for pulsars with small dipole moments. It has been suggested that the evolution of pulsars involves a decrease in the effective dipole moment. That would happen if the pulsar magnetic field decayed¹⁴ or realigned¹⁵, or if its configuration in the magnetosphere became more complicated¹⁶ as the pulsar grew older. Then, the pulsar evolutionary lines would be straight initially but would later deviate to lower values of \dot{P} , eventually becoming almost vertical. Again, it seems that the subpulses drift later while the magnetic dipole moment is strong, but change their direction of drift when it becomes weaker in the later stages of the lifetime of the pulsar.

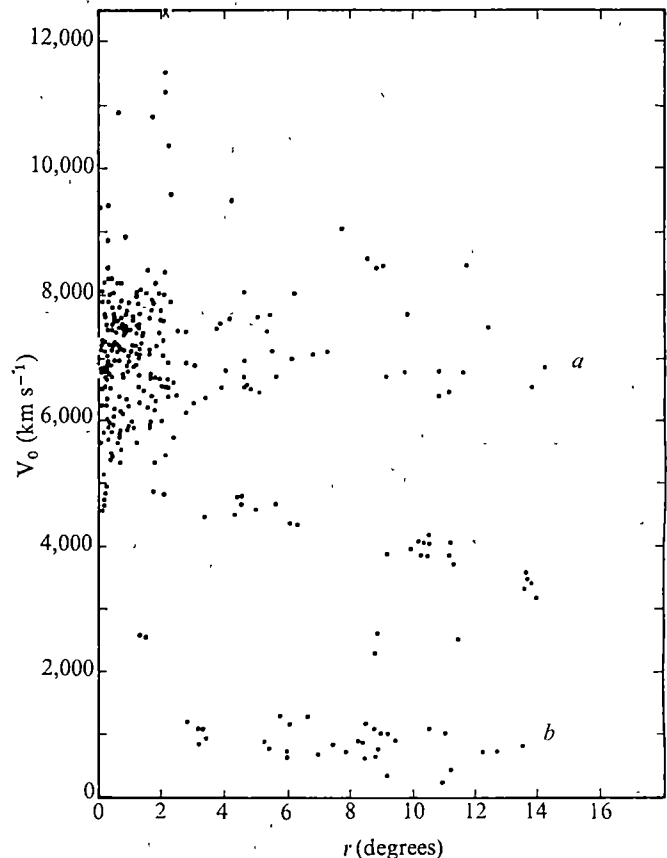
The relationship suggested here is based on the observation of a limited number of pulsars. Further observations are clearly required to test this apparent relationship, which may

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Size of the Coma cluster

STUDIES of counts of galaxies in large regions centred on the Coma cluster led Zwicky to conclude that the radius of the Coma cluster is at least 6° (13 Mpc for $H = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$). Outside this radius, the contribution to the counts from fore-

Fig. 1 Line of sight velocity relative to the Local Group against radial distance from the centre of the Coma cluster. *a*, Coma cluster; *b*, Local Supercluster.



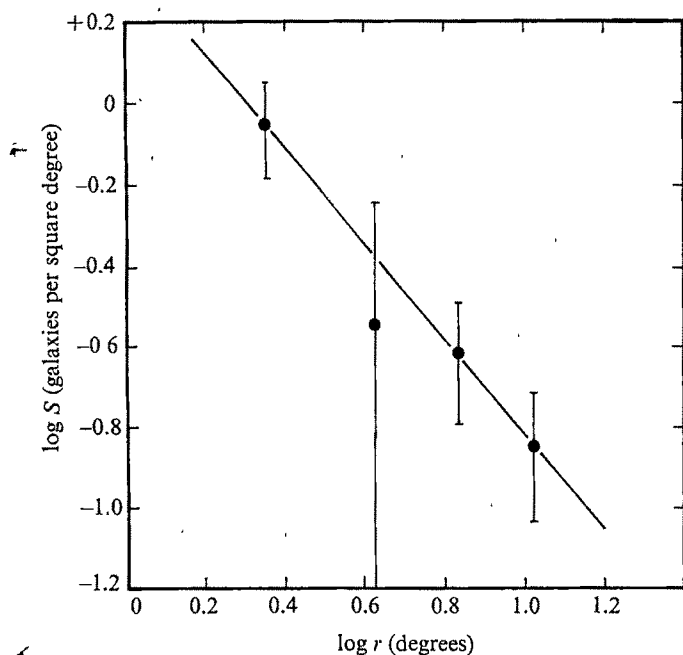


Fig. 2 Logarithm of the average number of galaxies in the Coma cluster with $m_p \leq 15.0$ mag per square degree against logarithm of the average radius of an annular ring. Error bars indicate the mean errors of sampling. The straight line has a slope of -1.18 .

ground and background galaxies overwhelms the contribution from the cluster, rendering it invisible. The technology of image intensifier tubes has now advanced to such an extent that it is feasible to obtain line of sight (radial) velocities of large numbers of galaxies in fields of a cluster. These velocities can be used to distinguish cluster members from foreground and background galaxies². Such studies have confirmed that the Coma cluster extends to at least 5° (ref. 3).

The reported study here, which completes work begun in 1972 (ref. 4), concentrates on a region $5-13^\circ$ west of the centre of the Coma cluster called cluster number 16 in field 158 by Zwicky and Herzog⁵. Spectrograms obtained with a Carnegie image tube on the 2.1-m telescope at the Kitt Peak National Observatory have been used to complete the determination of radial velocities for 50 of the 52 galaxies in cluster 16 with apparent magnitude $m_p \leq 15.0$ mag (a homogeneous sample). Some additional velocities of bright galaxies just outside the boundary of cluster 16 were also obtained. These data are used in conjunction with velocities for galaxies within 5° of the centre of the Coma cluster⁶⁻⁸ to plot radial velocity (relative to the Local Group), V_0 , against distance from the centre of the Coma cluster, r (Fig. 1).

Cluster 16 is clearly resolved into three components: (1) part of the Local Supercluster (V_0 between 200 and 1,300 km s⁻¹), (2) groups with V_0 between 2,200 and 4,200 km s⁻¹, and (3) the Coma cluster with velocities in the range 6,300–9,100 km s⁻¹. Galaxies in the Coma cluster with radial distance up to 14.2° are recognised, so the Coma cluster has been detected to $r = 32$ Mpc.

Seventeen galaxies (34%) of our homogeneous sample are cluster members. Fourteen of these can be categorised into morphological types⁹, and 7–8 (50–57%) are spirals. This fraction is consistent with the tendency for the relative number of spirals to increase with radial distance found for the inner 2.79° of the cluster⁶.

The data of our homogeneous sample have been combined with other homogeneous data⁶⁻⁸ to derive the average surface density (galaxies with $m_p \geq 15.0$ mag per square degree) against distance from the centre of the Coma cluster for the r interval $1.67-12.4^\circ$ (Fig. 2). A χ^2 fit of the data to a power law reveals that the surface density is proportional to $r^{-1.18 \pm 0.24}$, which

is consistent with the formula for an isothermal sphere found to apply in the inner regions of the cluster^{1,10-12}. If the empirical formula for surface density applies to even greater radial distances, then we expect 12% of a sample of galaxies with $m_p \leq 15.0$ mag at $r = 20^\circ$ (and 4.5% at 45°) to be members of the Coma cluster.

Our homogeneous sample, which is centred at $r = 8.5^\circ$, has a mean radial velocity of 7,639 km s⁻¹ and a standard deviation about this average of 751 km s⁻¹. The inner region, of the Coma cluster, radius 2.79° , has a mean radial velocity of 6,981 km s⁻¹ and a standard deviation of 920 km s⁻¹. The greater mean velocity of our homogeneous sample (by 2.4σ) relative to the inner region of the cluster may be a statistical fluctuation. If cluster members just outside the region of the homogeneous sample are included, then the average velocity reduces to 7,374 km s⁻¹.

An outer radius for the Coma cluster of at least 32 Mpc makes it one of the largest known cosmic entities. Its size is at least as great as the size of the Local Supercluster and typical groups of galaxy clusters (H.J.R., unpublished). A galaxy moving with a velocity of $900\sqrt{3}$ km s⁻¹ would take 2×10^{10} years to travel 32 Mpc. Thus gravitational mixing of galaxies between the inner and outer regions of the Coma cluster is not well advanced, and the properties of the outer regions reflect to a significant extent the initial conditions of the cluster formation period following the origin of the Universe.

The density of rich clusters of galaxies is of the order of 0.8×10^{-6} clusters Mpc⁻³. If the Coma cluster is a fairly typical cluster, as it seems to be^{13,14}, the new findings require a mass of $M(\text{Coma}) \geq 1.3 \times 10^{16} M_\odot$ and a density of the Universe $\geq 7.1 \times 10^{-31}$ g cm⁻³ = $0.12(3H_0^2/8\pi G) = 0.12\rho_c$.

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Possible origin of lunar magnetism

ANY model of the origin of lunar magnetism needs to explain two principal observations¹. One is the magnitude of the stable natural remanent magnetisation (NRM) carried by the returned lunar samples, which is far too large to have been acquired in fields comparable with those found at present in the vicinity of the Moon. The other is the presence of remanent fields at the lunar surface, which imply source regions of tens or hundreds of kilometres of homogeneous magnetisation, primarily in the highlands.

Attempts to explain these phenomena include the suggestion that the Moon had, early in its history, a regenerative dynamo like the Earth's, so that the magnetisation now observed was acquired in its field². It has also been proposed that the Moon

was exposed to an external field in which the observed magnetisation was acquired³, and Urey and Runcorn⁴ and Strangway and Sharpe⁵ have conjectured that the Moon was magnetised, more or less homogeneously, early in its history, and that the present magnetisation was acquired in this remanent field. The primitive remanent field was later thermally demagnetised as the Moon heated up internally. Finally, there are models in which local and transient fields are invoked. These models tend to merge into zero-field or 'bootstrap' models which require fields no larger than the present field associated with the solar wind. Here I present another possibility, which is a variant of the remanent field models already proposed.

I suggest that the Moon acquired a primitive magnetisation during its formation by accretion from planetesimals. Two mechanisms of magnetisation are likely to have been predominantly important. First, thermoremanent magnetisation would be acquired by material which was heated above its Curie point during impacts and subsequently cooled. Second, shock magnetisation would be acquired by material which was shocked but not heated significantly by the impacts⁶.

The nature of the fields in which the proposed magnetisation was acquired remains obscure. At an early stage of the Solar System an enhanced solar wind field is not, however, implausible. Also, because the mechanisms of thermoremanent and shock magnetisation are efficient, it is not necessary to propose as large a field as in the isothermal remanence model⁵. Finally, if these ideas are combined with those of A. E. Ringwood's recent models of the origin of the Moon (unpublished), it seems possible to invoke an early geomagnetic field as a source field for the primitive magnetisation of the Moon. In this view, the Moon accretes from a ring of planetesimals in a near-Earth orbit, so that if this process took place after the formation of the Earth's core, as seems likely, the terrestrial dynamo may already have been generating a geomagnetic field.

Whether an impact, or an external field origin is invoked, the primitive magnetisation of the Moon is likely to have been inhomogeneous. Its magnitude and direction would have been controlled by the geometries of impacts and of ambient fields. The latter would change with the proto-Moon's position in its orbit. In this respect, my suggestion differs from earlier models⁶ in that they propose a more or less homogeneously magnetised Moon. My explanation derives in part from an earlier suggestion made by Meadows⁷ to account for the magnetism of meteorites.

The chief difficulty with the remanent field approach lies in explaining any very strong NRM carried by the returned samples, which would imply inducing fields of the order of 1 oersted. It is not yet clear whether a significant fraction of the returned samples carry such NRM, so this may not be a critical difficulty. Nevertheless, if large numbers of samples do turn out to imply such strong inducing fields, the present suggestion will be seriously weakened. It will then be necessary to invoke somewhat *ad hoc* field amplification mechanisms to account for the high fields, compared with the fields of about $10^3\gamma$ which remanent field models seem capable of giving at the surface.

My suggestion follows the earlier remanent field models^{4,5} in the explanation of the loss of the primitive magnetisation by later internal heating of the Moon. The field could, however, be maintained by an early cool Moon at least long enough for the crust of the Moon to be magnetised when it was formed. This magnetisation of the crust would have been primarily a thermoremanent magnetisation (TRM), but shock magnetisation is also likely to have been involved since impacts took place while the field was present. A critical prediction is that the primitive magnetisation of the Moon was inhomogeneous on a Moon-wide scale, so that the magnetisation of the crust would also be inhomogeneous on the same scale. It certainly could, however, be homogeneous over scale lengths of hundreds of kilometres. This prediction can probably be tested by the magnetometer experiment on the Lunar Polar Orbiter.

If magnetisation by the process suggested here is important in the Moon, then it should also be so in other bodies of the

Solar System which were formed in the presence of magnetic fields. If the body remained cool, a record of the process should be preserved. If the body subsequently heated up internally, the magnetisation should have been lost and the surface record of the primitive magnetisation progressively obscured by later impacts and other effects. These may take place in very weak fields and thus demagnetise material, or in later dynamo fields, in which case new patterns of magnetisation will be generated.

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Recent crustal movements along Mediterranean coastal plain of Israel

THE high density of population along the Mediterranean coast of Israel dictates caution in the siting of power stations and major engineering structures. One of the critical questions is whether the coastal plain of Israel may be assumed to be tectonically quiet and stable. Lately¹, evidence was presented of Recent (probably later than 700 BP and certainly not earlier than 3,700 BP) faulting along the coast, and the coastline was assumed to be tectonic in origin, in contrast to the previously held notions of tectonic calm and a coastline shaped by wave abrasion^{2,3}. Though unable at this stage to comment on the regional implications of this controversy, we report that repeated precise levelling suggests the present occurrence of differential crustal movements along the coastal plain and the nearby Judea mountains.

Our data, based on 251 twice-levelled benchmarks aligned in seven complete and three incomplete polygons (Fig. 1), include the computed differences, between differences in elevation between consecutive benchmarks, determined in the 1962 and 1969 circuits in levelling ($dH = \Delta H_{1962} - \Delta H_{1969}$). Monumentation and geodetic operations conformed to specifications of first order precise levelling^{4,5}, discrepancy between the forward and backward measurement not exceeding $3\sqrt{D}$ mm (D is the levelled distance in km). The dH values are not related in sign nor magnitude to the discrepancies, and in most cases are significantly larger.

Figure 1 shows the locations of the studied benchmarks and the range of the computed dH values. Tide data are lacking and thus the dH values can not be related to the mean sea level. Instead, they are presented relative to a reference benchmark, with respect to which all other benchmarks subside (zero point in Fig. 1). The numbers shown on the map indicate in mm the lower and upper limits of the 1962–69 relative subsidence for each closed polygon. Arrows show the direction of tilt, and crosses indicate even subsidence relative to the reference benchmark.

Figure 1 indicates a pronounced regional tilt to the south-east and east-south-east. The differences between the adjacent polygons vaguely suggest a possible block-structure, related to the E–W trending normal faults revealed in gravity surveys of the coastal plain^{6,7}. Rate of movement and rate of tilting increase southwards. Polygons I, II, and III show a relatively gentle movement, the northernmost one subsiding evenly and

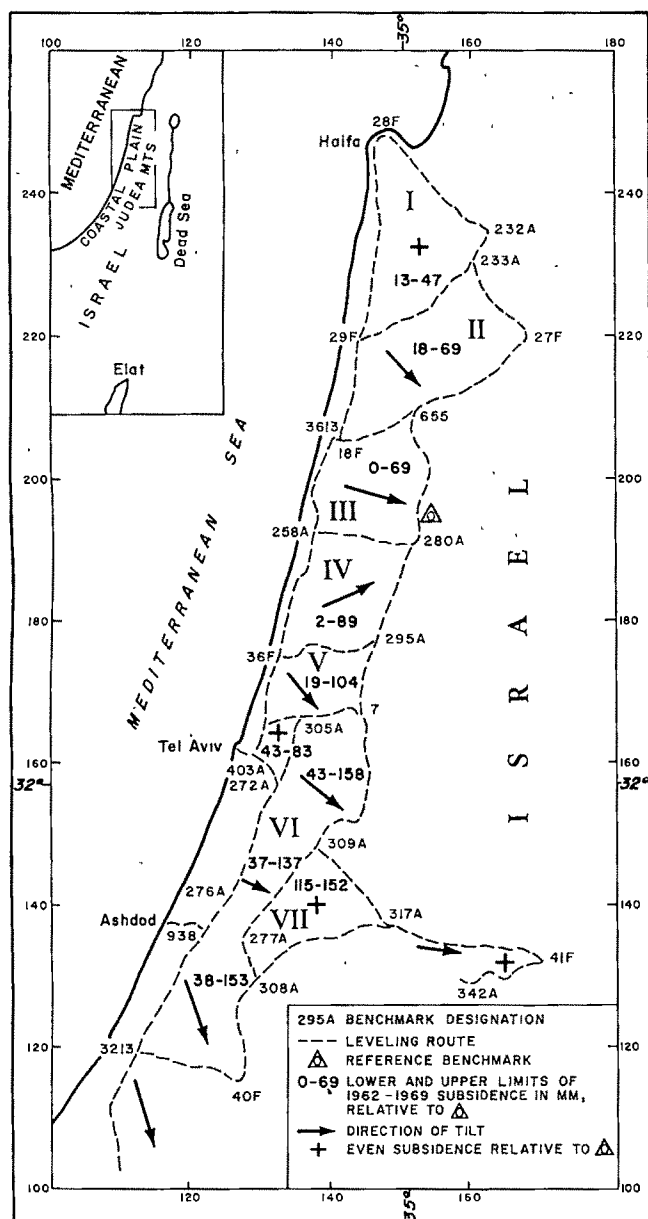


Fig. 1 Area of survey, showing variations in tilt.

the other two tilted towards ESE and SE. In polygons V and VI, tilts are stronger and the dH values reach 150 mm (an inferred movement of about 2 cm yr^{-1}). The strong easterly component of tilt suggests that the Judea mountains anticlinorium subsides relative to the coastal plain. This tendency of structural highs to sink, and of structural lows to rise, was reported also from other parts of the region⁸ and was tentatively attributed to gravity-compensation effects. It is not clear whether the southerly increase in rate of movement and tilt is tectonically controlled, or is caused by another factor such as differential loading due to a southerly increase in thickness of the sediments derived from the Nile^{9,10}.

Geological structure of the coastal plain is unexposed and structural details must be inferred from geophysical surveys and borehole correlations, thus disallowing a more detailed evaluation of the possible relationship between the dH movement of individual benchmarks and their structural setting.

The magnitude of inferred vertical crustal movements reported here is very considerable and must await verification by further levelling especially as only 25% of the benchmarks are anchored in bedrock. The time interval between the studied levellings is too short to reveal whether the dH values reflect a

consistent long term trend, or whether they are but a part of short term oscillations. But geological evidence of young tectonic activity in Israel reported in the past, and confirmed by seismological data^{11,12} indicates that the possibility of significant present day strain accumulation cannot be ignored.

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Influence of mantle CO_2 in the generation of carbonatites and kimberlites

THE explanation of why partial fusion of mantle peridotite sometimes yields basalts, sometimes kimberlites and sometimes carbonatites is given by the phase relationships in the system $\text{CaO-MgO-SiO}_2\text{-CO}_2$. The key is the intersection of a sub-solidus carbonation reaction with the solidus, which introduces carbonates as primary minerals alongside silicates on the surface of the liquidus.

The mineral assemblage forsterite (Fo) + orthopyroxene (Opx) + clinopyroxene (Cpx) in the system CaO-MgO-SiO_2 has been used by Kushiro¹ as a model for mantle peridotite, both dry and in the presence of H_2O , and by Eggler² in the presence of CO_2 . The P - T diagram of Fig. 1 compares the solidus curve for the mineral assemblage free from volatiles with the melting curves produced by solution of H_2O or CO_2 . At 20 kbar, the melting temperature is lowered through more than 400°C by the solution of about 20 weight % H_2O , and through 75°C by the solution of about 5 weight % CO_2 . The model mantle peridotite yields a haplobasaltic liquid that is forsterite-normative. In the presence of H_2O the liquid is enriched in SiO_2 and it is quartz-normative at 20 kbar (ref. 1). In the presence of CO_2 the liquid is depleted in SiO_2 , and somewhere between 15 and 30 kbar it becomes larnite-normative³, a haplokimberlite magma.

Our new data between 25 and 35 kbar show the solidus in the presence of CO_2 sweeping down through 400°C through a pressure maximum at about 32 kbar, and terminating at invariant point Q_6 near 25 kbar and $1,200^\circ\text{C}$. This is the point where the subsolidus carbonation reaction for the mantle mineral assemblage (Fig. 2) terminates by addition of a liquid phase. Increase in pressure not only stabilises carbonate in the subsolidus mineral assemblage, but also in the liquid phase; this produces a large increase in CO_2 solubility and a dramatic change in melting temperature.

Figure 2 is an isobaric isothermal diagram showing the compositions of the minerals involved in the subsolidus carbonation reaction at mantle pressures, with dots giving the compositions of pyroxenes and carbonates coexisting across solvi⁴⁻⁶. On the high pressure side of the reaction, the assemblage Fo + Opx + Cpx reacts to produce Cd (solid solution between calcite and dolomite) at the expense of Cpx. This reaction was reported in an

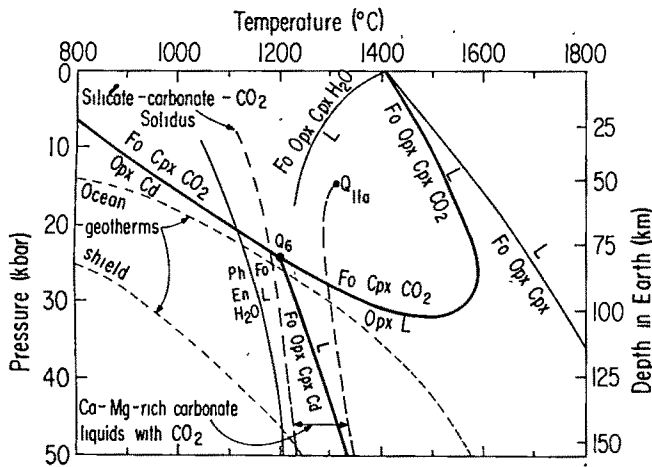
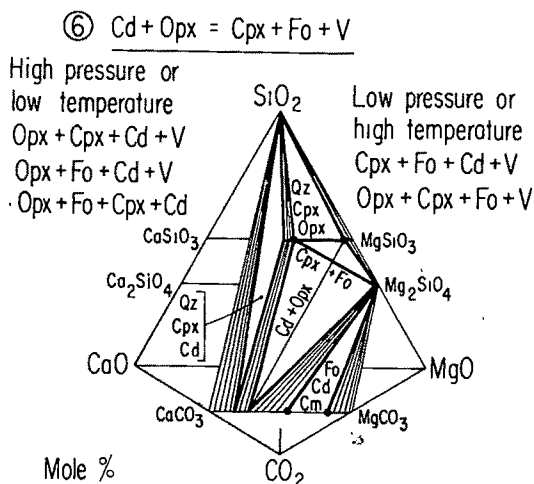


Fig. 1 The melting curve for mantle peridotite assemblage forsterite (Fo) + orthopyroxene (Opx) + clinopyroxene (Cpx), compared with reactions in the presence of H_2O , and with a small proportion of CO_2 , either as vapour phase at lower pressures, or as crystalline carbonate at higher pressures. Cd or Ccd = Solid solution between calcite and dolomite; L = liquid; Ph = phlogopite; En = enstatite. Of particular interest is the dramatic change in CO_2 -melting reactions between 25 and 35 kbar. (The numbering system, Q_6 , Q_{11a} , refers to a detailed account of the whole system, now in preparation.)

abstract by Eggler, but with a magnesian carbonate⁶. The solidus curve rising to higher pressures from Q_6 involves silicates and the carbonate. The first liquid produced is a haplo-carbonatite with about 90% by weight of carbonate.

Figure 3 shows the CO_2 -saturated liquidus surface in CaO - MgO - SiO_2 - CO_2 at 28 kbar (refs 5-8 and our unpublished work). For silicates in the area SiO_2 - $CaSiO_3$ - $MgSiO_3$, CO_2 solubility remains low, but for compositions ranging from the pyroxene join to the carbonate join, the CO_2 content of liquids increases significantly. Note the position of the field boundary between the silicates and the carbonate solid solutions, and the liquidus minimum at point m on this surface. This minimum (and related reactions at other pressures) is represented in Fig. 1 by the dashed line solidus at temperature slightly below Q_6 . This is close to the melting curve for phlogopite coexisting with mantle minerals and H_2O (ref. 9). Figure 1 shows the range of existence for haplo-carbonatites with compositions in the general area

Fig. 2 Isobaric isothermal diagram showing mineral compositions in the univariant carbonation reaction for the mantle mineral assemblage. This is the reaction terminating at invariant point Q_6 in Fig. 1, with the addition of a liquid phase. All phase assemblages shown coexist with CO_2 (V = vapour).



between m and a in Fig. 3. The solidus reaction without CO_2 rising to higher pressures from Q_6 would be represented in Fig. 3 by a point behind the liquidus surface, somewhat further away from the CO_2 apex than liquids m and a , but with similar high contents of dissolved carbonates.

At 28 kbar (Fig. 1) the melting reaction for the assemblage $Fo + Opx + Cpx + CO_2$ is intersected twice, and the liquid compositions are represented in Fig. 3 by the higher temperature liquid b (haplokimberlite) and the lower temperature liquid a (haplo-carbonatite). With increasing pressure, points a and b approach each other, becoming coincident at about 32 kbar (Fig. 1). At greater pressures, the CO_2 -saturated liquidus fields for forsterite and clinopyroxenes are separated by the field for orthopyroxene.

The peridotite carbonation reaction in Figs 1 and 2 occurs at lower pressures than the forsterite carbonation reaction in the system MgO - SiO_2 - CO_2 as determined by Newton and Sharp¹⁰. According to the geotherms¹¹ and the reaction in Fig. 1, we confirm their conclusion that free CO_2 cannot exist in the upper mantle except in conditions of unusually high temperature. CO_2 exists in the upper mantle as carbonate^{10,12}. The reaction is displaced to lower temperatures in the presence of diluted

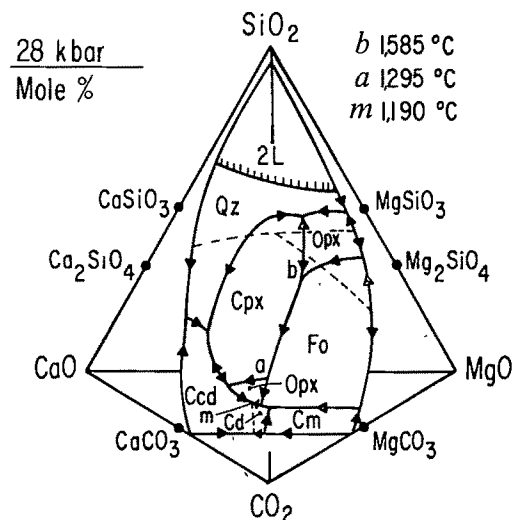


Fig. 3 CO_2 -saturated liquidus surface in CaO - MgO - SiO_2 - CO_2 at 28 kbar, partly schematic. The surface gives the compositions of liquids that can coexist with free CO_2 , and shows the fields for primary crystallisation of minerals. Cm, Cd, and Ccd are carbonate solid solutions; Qz = quartz; 2L = immiscible liquids. Note the continuous crystallisation or fusion paths between haplo-basalt (liquids in area olivine-pyroxenes), haplokimberlite, liquid b , and haplo-carbonatite, liquid a .

CO_2 , however, so CO_2 could exist within a restricted depth interval in a CO_2 - H_2O vapour phase, probably of limited compositional range.

The seismic low velocity zone may be caused by incipient melting due to the presence of traces of H_2O in the upper mantle^{13, 14}. Green concluded that melting was not involved, and that the low seismic velocities were caused by exsolution of CO_2 from dry silicate minerals^{15,16}. Figure 1, however, shows that mantle peridotite with CO_2 must begin to melt in the depth range 80-130 km.

In the presence of CO_2 , partial fusion of mantle peridotite at depths shallower than 80 km produces basaltic magmas, which become more undersaturated with silica at increasing depth². At depths greater than 80 km, the first magmas produced, in small quantities, are carbonatites. With progressive fusion these are converted to kimberlites and eventually, at higher temperatures, to basalts.

Any of these magmas, with fractional crystallisation at constant pressure, can yield carbonatite as residual magma at

considerably lower temperatures^{6,7}. Kimberlites or carbonatites rising from the low velocity zone must evolve CO₂ as they reach the reaction boundary at depths between about 100 and 80 km. This would certainly contribute to the explosive emplacement of kimberlites.

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Palaeogeothermal gradients derived from xenoliths in kimberlite

GREAT problems exist in the determination of an ancient geothermal gradient. An attempt has been made by Boyd¹, based primarily upon the equilibration conditions of co-existing pyroxenes in garnet lherzolite xenoliths from the Thaba Putsoa, Letseng and Mothae kimberlite intrusions in northern Lesotho. He derived the equilibration temperature of the xenoliths from the amount of enstatite in solid solution in the diopside², and estimated the pressure from the amount of Al₂O₃ in the orthopyroxene³. He argued that, for a group of lherzolite xenoliths, a line joining a series of pyroxene-derived pressure/temperature points should define the geothermal gradient in the mantle below northern Lesotho during the Cretaceous when the xenoliths were entrained by the kimberlite during its ascent. He found a Cretaceous gradient similar to that in present-day shield areas between depths of 100 and 150 km, though from 150 to 200 km depth it was considerably steeper. Moreover, whereas the lherzolites outlining the shallower 'normal' part of the gradient are of granular texture, those outlining the high-temperature, steeper and deeper segment exhibit a range of deformation and recrystallisation textures (the porphyroclastic and mosaic textures in the classification scheme of Bouillier and Nicolas⁴).

Accordingly, it has been proposed^{1,5} that the perturbation of the normal subcratonic gradient was caused by stress heating at the base of the African Plate during the breakup of Gondwanaland in the Cretaceous, with the 150 km inflection point on the gradient being the top of the sheared, low-velocity zone at that time. Inflected geothermal gradients have been proposed within the upper mantle beneath other parts of the South African Shield, the North American Craton and the Siberian Shield, based on granular and deformed peridotite suites from Jagersfontein⁶; Louwrencia⁷, SW Africa; Black Butte diatreme, Montana⁸; Ming Bar diatreme, Wyoming⁹; and the Udachnaya diatreme, Yakutia¹⁰. These data cannot, however, be accepted unreservedly as

typical of events within the upper mantle at the time of the generation and ascent of the kimberlite magma. There is evidence to the contrary in xenolith suites from at least three different kimberlite intrusions.

The peridotites and pyroxenites from the Matsoku kimberlite diatreme, Lesotho, have been more thoroughly investigated than any other xenolith suite¹¹⁻¹³. They show a wide variety of textures, including those falling into the 'sheared' class of Nixon and Boyd⁸. But no matter what degree of deformation the Matsoku xenoliths have undergone, they all apparently equilibrated under the same pressure/temperature (PT) conditions and there is thus no correlation between deformation and increased temperature of equilibration in this particular xenolith suite. The restricted PT conditions indicated are those of Boyd's¹ coarse granular suite. As the Matsoku kimberlite is closely contemporaneous with the Thaba Putsoa kimberlite, and is only 20 km from it, it is difficult to accept a hypothesis involving large-scale horizontal movements in the upper mantle that did not have similar effects at both localities.

In the case of the xenolith suite from the Frank Smith Mine, north of Kimberley, a variety of deformation textures can be found in rocks apparently derived from a restricted mantle zone¹⁴, although the range does not include granular textures as it does at Matsoku, and the equilibration temperatures are close to 1,250 °C.

We have been investigating a suite of xenoliths from the Bultfontein kimberlite diatreme, Kimberley. The garnet lherzolites show a greater textural range than those from Thaba Putsoa and we have recognised a new textural type that indicates a greater degree of deformation than recognised previously in kimberlite xenoliths. This new texture, which may be regarded as a more deformed extension of Bouillier and Nicolas' fluidal mosaic texture¹⁵, comprises recrystallised orthopyroxene neoblasts strung out into bands that alternate with bands of fine-grained olivine neoblasts; furthermore, garnet unaffected by lower degrees of deformation has been disrupted and strung out into chains of small crystals. We call this texture 'banded and disrupted' (BAD).

We have analysed the phases in 26 selected garnet lherzolites from Bultfontein, covering the range of textural types from granular to BAD. The data will be published elsewhere but Table 1 shows analyses of phases in typical

Fig. 1 Plot of Al₂O₃ (in orthopyroxene) against Ca/Ca + Mg (in clinopyroxene) for xenolith suites from Thaba Putsoa⁵ (open symbols) and Bultfontein (solid symbols). Triangles, minerals in rock of granular texture; circles, minerals in rocks of flaser (Bultfontein) and sheared (Thaba Putsoa) texture. The Bultfontein flaser peridotites exhibit porphyroclastic, mosaic and BAD textures.

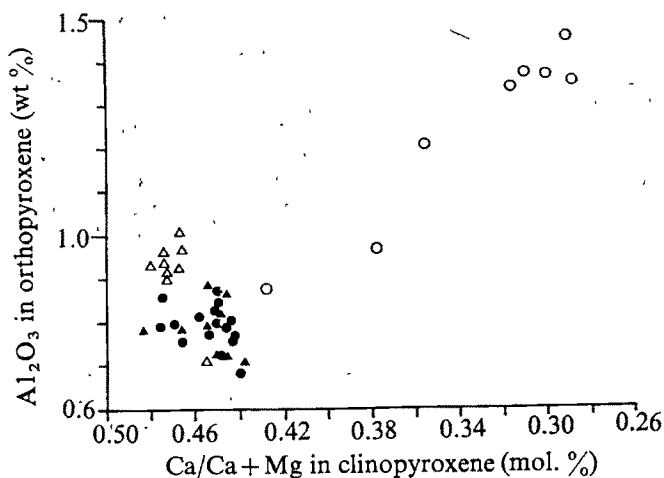


Table 1 Analyses (a) and structural formulae (b) of minerals in typical Bultfontein peridotites at opposite ends of the deformation spectrum

a	BD2382: granular garnet lherzolite				BD2446: banded and disrupted garnet lherzolite			
	Olivine	Orthopyroxene	Clinopyroxene	Garnet	Olivine	Orthopyroxene	Clinopyroxene	Garnet
SiO ₂	40.00	57.38	54.79	41.69	41.51	57.05	54.83	41.93
TiO ₂	0.02	0.04	0.11	0.08	0.00	0.05	0.07	0.05
Al ₂ O ₃	0.01	0.78	2.39	22.62	0.02	0.82	2.51	20.67
Cr ₂ O ₃	0.00	0.06	1.42	2.19	0.02	0.36	2.53	5.01
FeO*	8.87	5.58	2.51	8.19	6.88	4.22	1.88	6.92
MnO	0.04	0.03	0.00	0.36	0.00	0.09	0.07	0.36
MgO	50.96	36.14	16.27	22.62	51.41	36.48	16.54	20.34
CaO	0.02	0.27	21.26	4.78	0.01	0.31	18.94	5.05
Na ₂ O	0.00	0.10	2.00	0.02	0.06	0.09	2.27	0.05
K ₂ O	0.00	0.00	0.00	0.00	0.02	0.01	0.02	0.00
Total (wt %)	99.9	100.4	100.7	98.5	99.9	99.5	99.6	100.3
b								
Si	0.979	1.965	1.972	3.022	1.003	1.961	1.982	2.992
Ti	0.000	0.001	0.003	0.004	0.000	0.001	0.002	0.003
Al	0.000	0.031	0.100	1.933	0.001	0.033	0.107	1.732
Cr	0.000	0.002	0.040	0.126	0.000	0.010	0.072	0.282
Fe	0.181	0.160	0.076	0.540	0.139	0.121	0.057	0.413
Mn	0.001	0.001	0.000	0.022	0.000	0.006	0.002	0.022
Mg	1.859	1.845	0.873	1.925	1.852	1.872	0.892	2.164
Ca	0.001	0.010	0.820	0.371	0.000	0.011	0.732	0.386
Na	0.000	0.007	0.140	0.003	0.003	0.006	0.159	0.007
K	0.000	0.000	0.000	0.000	0.001	0.001	0.001	0.000
O	4	6	6	12	4	6	6	12
Mg/Mg+Fe	0.911	0.920	0.920	0.781	0.930	0.939	0.939	0.831
Ca/Ca+Mg			0.484				0.451	

*All iron as total FeO.

rocks from the two ends of the deformation spectrum. For the suite as a whole there are three pertinent features. First, no matter what the texture, the Ca/Ca+Mg ratio of the clinopyroxene is in the range 0.422–0.484 (wt %), indicating equilibration temperatures of 950–1,050 °C (ref. 2). Second, the Al₂O₃ content of all the orthopyroxenes is very similar to that in lherzolite orthopyroxenes in the Matsoku kimberlite (0.73–0.89 wt %), again indicating equilibration over a restricted pressure (depth) interval (Fig. 1). In view of the absence of experimental data on orthopyroxenes containing less than 2 wt% Al₂O₃, we are not prepared to draw any pressure (depth) implications from our results, other than to submit that they are of mantle origin, within the garnet lherzolite stability field. Third, the composition of the garnets is broadly similar in most lherzolites, with the exception that in a very few of the more deformed lherzolites, there are small increases in the TiO₂ content.

These data provide evidence that increased deformation cannot always be correlated with significant increases in temperature of equilibration or with increased depth or origin, as is the case for Thaba Putsoa xenoliths. Further, we have observed deformation gradients from granular to BAD textures within the same hand specimens over a distance of 2–3 cm and must assume that the deformation may be both very localised and very variable. This is not consistent with a major tectonic boundary, and may be caused by variable hydrolitic weakening.

It is clear that within the xenolith suite from each locality there is evidence of major deformation, consistent with differential movements in the upper mantle. One feature that is not clear is the sense of these movements. Strong lateral movements, accompanying the breakup of Gondwanaland, are proposed in the Thaba Putsoa model of Nixon and Boyd⁴, whereas the model for the origin of kimberlites by diapiric upwelling in the upper mantle¹⁶, invokes strong vertical movements. The evidence of individuality in the range of textures and equilibration conditions of xenolith suites from different diatremes is possibly more consistent with the latter hypothesis in that it links the characteristics of a xenolith suite with the particular upper mantle movements that culminate in the

emplacement of the kimberlite intrusions. Nonetheless, we do not exclude the possibility that some of the textures developed during the kimberlite event may be additional to textures developed during earlier upper mantle creep. A model involving vertical movement finds support in the geological evidence in that, during the Cretaceous, when most South African kimberlites were emplaced, the South African continent was subjected to strong vertical movements, resulting in peripheral faults downthrowing 18,000 m towards the contiguous ocean basins¹⁷.

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Morarian Orogeny and Grenville Belt in Britain

Now that the nature of the Morarian orogenesis (about 730 Myr BP) is becoming more apparent^{1,2} it seems appropriate to assess its significance in terms of late Proterozoic history of the northern Atlantic region. It has been argued³ that the event is related to subduction, with a suture partly coinciding with the Great Glen Fault. The event has also been associated with the closing of a Precambrian ocean during the time represented by the unconformity between the Stoer and Torridon groups⁴. The Proto-Atlantic opened after the deposition of the Torridon Group. In establishing the orogenic character of the Morarian Event several features are important. First, the event is known to have affected Moine rocks in a zone some 20 km wide. If, as seems likely, the Morarian fabric can be traced as far south as the Great Glen Fault then the belt is some 50 km wide after the Caledonian shortening. Until more is known about the geology of Sutherland, however, it is uncertain whether the entire length of the Moine outcrop (about 250 km) is involved. There is no reason to believe that the Morarian fabric is present to the south of the Great Glen Fault: the likelihood is that the Central Highland Granulites (often named the Moines nowadays) represent post-Moine sediments, but the possibility that they are Moines that were not influenced by the Morarian Orogeny cannot be excluded. Until this problem is resolved it would be prudent to revive the name Central Highlands Granulite for the sub-Dalradian rocks occurring to the south of the Great Glen Fault.

Second, the Morarian belt is an Alpine-type belt. At Glenelg, Lewisian basement has been tightly folded with the Moine cover. Comparable structures may be present in Sutherland where sheets (nappes) of Lewisian gneiss mark a continuation of the Sgurr Beag Slide⁵. In contrast to the Sgurr Beag Slide the tectonic features responsible for these sheets are marked by a substantial number of ultrabasic bodies (serpentines, scyellites, garnet-pyroxene rocks)⁶, which have penetrated the Lewisian and the surrounding Moines. These bodies are, therefore, synchronous with or later than the emplacement of the sheets. There is some evidence that the serpentines antedate the D2 deformation (Caledonian)⁷. It is conceivable that the Lewisian nappes in Sutherland belong to the Morarian Orogeny.

Third the Morarian Belt is asymmetrical. The vergence of the Morarian antiforms is eastwards⁸ in contrast to the westerly translation of the Caledonian structures in north-western Scotland. The 'root zone' of the Morarian antiforms is exposed in the Glenelg region where the western part of the zone has been truncated by the Moine Thrust. If the Lewisian sheets in Sutherland are Morarian nappes rooted to the west then the Moine Thrust in northern Scotland has cut off the root zone. The implication is that the root zone lies wholly or in part beneath the Moine Thrust.

Fourth, the Morarian Orogeny was associated with upper amphibolite facies metamorphism resulting in the development of migmatites on a regional scale. In spite of the apparent lack of igneous rock which can be related to the Morarian Event there are some grounds for regarding the Morarian as an orthotectonic belt.

Fifth, there is no obvious connection between the Morarian Orogeny and the Stoer and Torridon groups which seem to antedate it. Thus, neither of these groups can represent molasse and, setting aside the possibility that the Central Highland Granulites is such, there is no identifiable sedimentary deposit that can be linked with any uplift of the Morarian Orogen.

These considerations allow speculation about a plate-tectonic model in the context of the late Proterozoic history of the northern Atlantic region. Garson and Plant³ have inferred the presence of a subduction zone, active about 730 Myr BP, following the ultrabasic zone in Sutherland and along the line of the later Great Glen Fault. That hypothesis encounters, however, several difficulties⁷. The ultrabasic bodies are emplaced at a high level in the structural pile: they must have penetrated some 2–3 km of Moine rocks to reach their present position. Accordingly, I interpret them as high level diapirs, derived from oceanic lithosphere, which, of course, are not situated on a subduction zone. The geographical association of these bodies with Lewisian sheets is probably significant and suggests a tectonic control (a zone of sliding) for their emplacement. Another possible difficulty is that the model devised by Garson and Plant³ implies that the "Moine" and Dalradian rocks of the Grampian Highlands were deposited on oceanic crust situated to the south of the proposed subduction zone.

As an alternative hypothesis, I suggest that the Morarian suture is hidden beneath the Caledonian nappes, that is, the Glenelg and Rossshire nappes. This follows from the vergence of, and the westerly root for, the Morarian nappes. A further prospect is that the Morarian is a collision-type belt, and the implication of that (see ref. 8) would be that the Moines were deposited on one continent (the Baltic Shield or a microcontinent) and the Stoer and Torridon groups were deposited on another—the Laurentian.

If the portrayal of the Morarian as an orogenic event is correct then it is worth considering its setting in the wider context. On present evidence the event seems to have been localised: apart from in Spitzbergen⁹ and western Ireland¹⁰ there is no firm evidence from the northern Atlantic Region of orogenic activity at 800–700 Myr BP. It may be that the Morarian Event is commonly obscured, to an even greater degree than it is in Scotland, by strong Caledonian overprinting, and in this respect anomalous radiometric dates, such as the 700–800 Myr dates recorded in the Appalachians¹¹, are worth re-examination.

But assuming that the Morarian event was localised, the likely hypothesis is that it resulted from the closure of a small ocean basin before the episode of rifting that formed the later Proto-Atlantic⁴. Another possibility is that the Morarian Event represents a delayed closure of the Grenville Ocean in the region of Britain, thus forming the missing link in Britain between Canada and Scandinavia^{12,13}. Orogenic events took place at widely different times during the closure of the Proto-Atlantic and adjacent oceans, and incomplete suturing produced the Erian, Acadian and Taconic episodes⁴. Though the time span between the Grenville and the Morarian events is substantially greater than that separating the Taconic and Acadian episodes of the Caledonian Orogeny, it should be borne in mind that the dated Morarian pegmatites may only date the end of the Morarian Orogenic Cycle.

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Magnetic properties of oceanic pillow basalts from Macquarie Island

We summarise here the results of a combined palaeomagnetic, hysteresis, thermomagnetic, and electron microprobe analysis of late Tertiary oceanic pillow basalts from Macquarie Island, which indicate that pillow basalts from a significant depth in the oceanic lithosphere have suffered burial metamorphism during the crustal accretion process. This metamorphism results in an intensity of natural remanent magnetisation (NRM) of $\sim 10^{-3}$ gauss, a substantial component of viscous remanent magnetisation (VRM), and an order of magnitude decrease in the Koenigsberger ratio relative to unaltered pillow basalts. Since a pillow lava layer with such a low NRM intensity is insufficient to account for the observed amplitude of marine magnetic anomalies, we conclude that in the case of Macquarie Island the underlying basaltic and doleritic dyke complex must also contribute to the observed anomalies.

Our data suggest a model in which the contribution to magnetic anomalies by the pillow basalt layer decreases with the age of the oceanic crust, though the contribution from the underlying dyke complex may not be susceptible to decay¹. The data also suggest that the credibility of the pillow basalt layer as the entire source of marine magnetic anomalies in old oceanic crust (at least 30 Myr BP) must be re-examined.

Field geological, petrological, geochemical, and marine geophysical data indicate that Macquarie Island (54° 15'S, 159°E) is an uplifted segment of late Tertiary (~ 30 Myr old) oceanic crust which was formed by seafloor spreading of the Australia–Antarctic Ridge^{2,3}. Pillow lavas exposed in the North Head region of Macquarie Island are relatively fresh and have not undergone burial metamorphism; however, pillow basalts exposed near Langdon Point have undergone significant burial metamorphism. Varne and Rubenach² have argued that this metamorphism is characteristic of the crustal accretion process at spreading oceanic ridges and is not the result of a metamorphic event associated with the tectonic uplift of Macquarie Island. This evidence indicates that the North Head exposures are representative of the upper (100–200 m?) portion of the oceanic basalt layer, whereas the Langdon Point lavas are from deeper oceanic crust ($> 1/2$ km deep?). To investigate the effects of burial metamorphism and seafloor weathering, oriented core samples were collected at 4 sites (nos 8–11) near Langdon Point and at 4 sites (nos 12–15) on the eastern coast of North Head.

Because of tectonic disturbance, NRM directions cannot be used for palaeomagnetic pole determinations but are very

useful in determining the presence of stable and viscous remanence at the sampling localities. The important mean parameters of natural remanence observed at both Macquarie Island sampling localities are summarised in Table 1. Directions of NRM for the Langdon Point sites show streaking toward the present direction of the axial dipole field at the sampling locality. On alternating field (a.f.) demagnetisation, the directions move away from the axial dipole direction. These observations indicate a significant component of viscous remanent magnetisation (VRM) in these metamorphosed pillow lavas. Experiments of the VRM acquisition confirmed the indication of a large viscosity coefficient, S , in samples from this locality. Directions of natural remanence at North Head sites show only a small tendency to streak towards the axial dipole field. Experiments on the acquisition of a.f. demagnetisation and acquisition of VRM also indicate that the unmetamorphosed basalts from North Head are not susceptible to large components of viscous remanence.

The geometric mean intensity of the NRM at both Macquarie Island sampling localities is surprisingly low. The geometric mean intensity is 0.69×10^{-3} gauss for samples from North Head and 0.57×10^{-3} gauss for Langdon Point samples (Table 1). In contrast, NRM intensities of some dredged samples have exceeded 10^{-1} gauss, and deep-tow magnetometer profiles over oceanic ridges also indicate a strongly magnetic layer of pillow lava in young oceanic crust^{4,5}. If the NRM intensity of these young pillow basalts does not decrease with depth into the oceanic crust, a thin (< 1 km thick) layer of strongly magnetic pillow basalts could account for the entire amplitude of marine magnetic anomalies. Seafloor weathering, however, causes low temperature oxidation of the magnetic minerals resulting in a decreased intensity of NRM^{6,7}. Data collected during the Deep Sea Drilling Project (DSDP) indicates that the upper pillow basalts of ancient oceanic crust have NRM intensities of $\sim 10^{-3}$ gauss^{8,9}. These values are in close agreement with NRM intensities of Macquarie Island pillow basalts, and are, perhaps, typical of old (> 30 Myr) oceanic crust in general. If that is the case, then the contribution of the pillow basalt layer alone is inadequate to account for magnetic anomalies observed over old oceanic crust.

From measurements of low field susceptibility, χ , the Koenigsberger ratio, Q ($= \text{NRM}/(\chi)(0.65 \text{ oersted})$), was determined for each sample. The mean Q value for North Head samples is similar to that observed in Atlantic DSDP basalts. The Langdon Point specimens show, however, a very high susceptibility and, consequently, a low Koenigsberger ratio. This high χ and low Q for Langdon Point samples indicates a difference in magnetic mineralogy between these metamorphosed pillow basalts and the unmetamorphosed pillows from North Head. Hysteresis properties for North Head samples indicate a distribution of magnetic grains which is predominantly within the single-domain range (R. F. B., S. K. B. and J. H. S., unpublished). By contrast, the hysteresis properties for Langdon Point samples indicate a substantial multi-domain (MD) content. This higher MD content would explain the higher magnetic viscosity coefficients in the Langdon Point samples.

Results of thermomagnetic analyses also indicate an important difference in magnetic mineralogy between the two Macquarie Island sampling localities. Irreversible thermo-

Table 1 Average parameters of remanent magnetism

Locality	Natural remanence (10^{-3} gauss)	Low field susceptibility (10^{-3} gauss oersted ⁻¹)	Koenigsberger ratio	Viscosity coefficient (10^{-5} gauss oersted ⁻¹)*
Langdon point (sites 8–11)	0.57	2.80	0.31	4.3
North Head (sites 12–15)	0.68	0.11	9.8	1.2

*Time measured in minutes.

magnetic curves were observed for all samples from North Head. An initial Curie temperature of between 300 and 400 °C was followed by an increase in magnetisation and a subsequent final Curie point between 500 and 580 °C. The saturation magnetisation after thermal cycling was always greater than the initial saturation magnetisation. This type of irreversible thermomagnetic behaviour has been observed in dredged pillow lavas⁷ and in DSDP basalts^{8,9}. The thermomagnetic results—and microprobe examinations of the opaque minerals (R. F. B., S. K. B. and J. H. S., unpublished)—indicate that the magnetic minerals in the North Head samples are non-stoichiometric titanomaghaemite. These cation-deficient phases are the products of low temperature oxidation resulting from prolonged seafloor weathering of the late Tertiary oceanic pillow basalts.

All of the Langdon Point samples show reversible thermomagnetic curves with a single Curie temperature very near 580 °C, indicating that the magnetic mineral in these metamorphosed pillow lavas is magnetite containing little or no titanium. Reversible thermomagnetic curves indicating Curie points of 580 °C have not been observed previously in analyses of dredged pillow basalts or DSDP basalts. This 'unusual' behaviour is almost certainly the result of burial metamorphism undergone by the Langdon Point basalts. The fine-grained titanomagnetites in young dredged pillow basalts are known to be very susceptible to chemical change when heated. At any significant depth in the oceanic crust pillow basalts would be exposed to heating by the intrusion of feeder dykes leading to overlying pillow lavas, the emplacement of underlying sheeted dyke and plutonic complexes, or even by exothermic hydration reactions within the metamorphosed pillow lavas themselves². Thus, the metamorphosed pillow lavas from Langdon Point and their resulting magnetic properties are thought to be the natural consequence of burial metamorphism experienced during seafloor spreading by basalts at significant ($> 1/2$ km?) depths in the oceanic crust.

The evidence from DSDP basalts, and the data presented here on pillow lavas from the North Head region of Macquarie Island indicate that the progressive submarine weathering of the oceanic crust decreases the NRM intensity of the upper portion of oceanic crust to $\sim 10^{-3}$ gauss. Lowrie, *et al.*⁸ have suggested that the 10^{-3} gauss NRM intensities of DSDP basalts are perhaps only representative of the strongly weathered zone at the top of the pillow lava layer and that unweathered pillow lavas below the weathered zone may be more strongly magnetised. But if the pillow lavas from Langdon Point on Macquarie Island are representative of pillow basalts from depths of 500–1000 m, the NRM intensity of these deeper extrusives would also be of the order of 10^{-3} gauss. Since a 1-km thick layer of pillow basalts magnetised at 10^{-3} gauss is not sufficient to yield the observed amplitude of marine magnetic anomalies, we conclude that the underlying basaltic and doleritic sheeted dyke complexes also contribute to the anomalies. Detailed analysis of changes in marine magnetic anomaly profiles with age of the underlying oceanic crust has led to a similar conclusion (R. J. Blakely, unpublished).

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Effect of oxygen on aldolisation reactions

ALTHOUGH the kinetics of aldolisation reactions have been studied^{1,2}, no observations have been reported concerning the effect of oxygen on their rates and kinetics. We have studied polarographically the aldolisation of acetaldehyde in 1 M lithium hydroxide in the absence of oxygen and spectrophotometrically in the presence of air using absorbance at 230 and 277 nm. A difference in the concentration–time curves obtained using these two procedures was observed.

The base-catalysed autoxidation of ketones is probably³ initiated by the interaction of molecular oxygen with carbanions formed in alkaline media and in the past the observed discrepancy has, therefore, been attributed to the interaction of atmospheric oxygen with carbanions formed as intermediates in the course of aldolisation.

To test this hypothesis, we studied reactions of α -phenyl-substituted aldehydes of the type $RCH(C_6H_5)CHO$ in alkaline media. The pK values of these compounds⁴ are such that they exist in 1 M lithium hydroxide almost completely in the carbanion form. Observations of these compounds during kinetic runs showed that they behaved differently in the presence and absence of oxygen. In the absence of oxygen, polarographic and spectrophotometric measurements gave identical rates. As a side product of the reactions $RCOC_6H_5$ compounds are formed in the presence of air⁵, further confirming the reaction of carbanion with oxygen. The latter reaction has also been postulated as a side product of the oxidation of phenylacetaldehyde to benzoic acid⁶ and in association with the alkaline rearrangement of aconitine⁷.

Thus some discrepancies reported for the kinetics of aldolisation may result from an interaction between molecular oxygen and the carbanion intermediate. Consequently, in future studies of aldolisation and other reactions which involve carbanion formation, oxygen should be excluded completely.

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Polymer drag reduction in Taylor vortices

THE Toms effect¹, whereby the addition of small quantities of polymer can typically produce 50% reduction in drag, occurs in turbulent flow systems. We report preliminary observations of a similar drag reduction behaviour observed in a Couette apparatus when toroidal Taylor vortices² are generated by rotating the inner of two concentric cylinders at sufficient speed; in this case the flow field is well understood and does not vary with time.

Simple shear alone as generated by normal Couette flow has little effect on deforming flexible polymer molecules and can be expected to exhibit no drag reducing effect; however, extensional flows such as pure shear, axial extension and compression do have the ability to extend molecules and the extensional viscosity when polymers are present may be orders of magnitude greater than the shear viscosity³. If this class of flow is present significant effects can be expected when polymers are added. In Taylor vortices the existence of extensional flow regions between each counter rotating vortex has already been

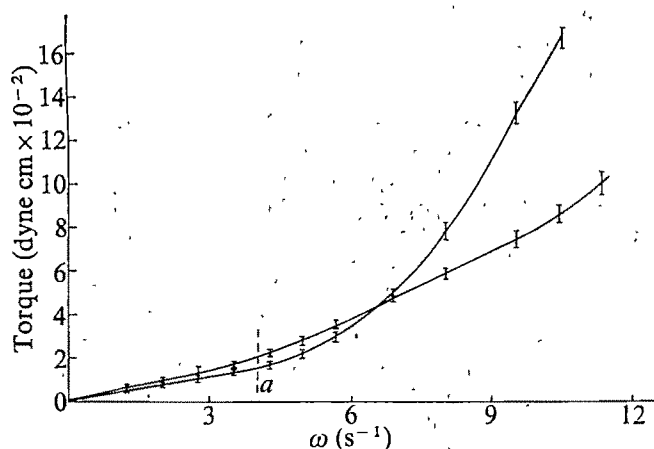


Fig. 1 Graph of torque as a function of angular velocity for a Couette apparatus 30 cm long, of inner cylinder diameter 2.0 cm and gap width 0.37 cm.

identified⁴ as the cause of nucleation of extended chain polymer crystals.

With a Couette apparatus we have measured the torque on the stationary outer cylinder using a fine torsion wire suspension. Figure 1 shows the torque measured for different rates of rotation of the inner cylinder using both water and polymer doped solutions. The polymer was polyethylene oxide (Union Carbide WSR301) of molecular weight $M_w \approx 4 \times 10^6$. The solution was renewed before each series of measurements to minimise the effect of possible shear induced degradation. For a low angular velocity of the inner cylinder when the flow is simple shear, the torque against ω curve obeys an essentially linear relationship for both solutions where the gradient of the line gives the shear viscosity, which is about 20% higher for the 50 p.p.m. polyethylene oxide (WSR301) water solution. At a critical speed (a in Fig. 1) the torque starts to increase non-linearly. This speed corresponds well with that predicted by Taylor² at which the secondary Taylor vortex pattern develops. Above this critical speed we assume that subsequent differences in the measured torque curve from the extrapolated straight line represent the additional drag caused by the secondary Taylor vortex flow. When Taylor vortices are well developed we see that the drag as measured by the torque is significantly reduced when polymer is present.

From our torque measurements we cannot say with precision

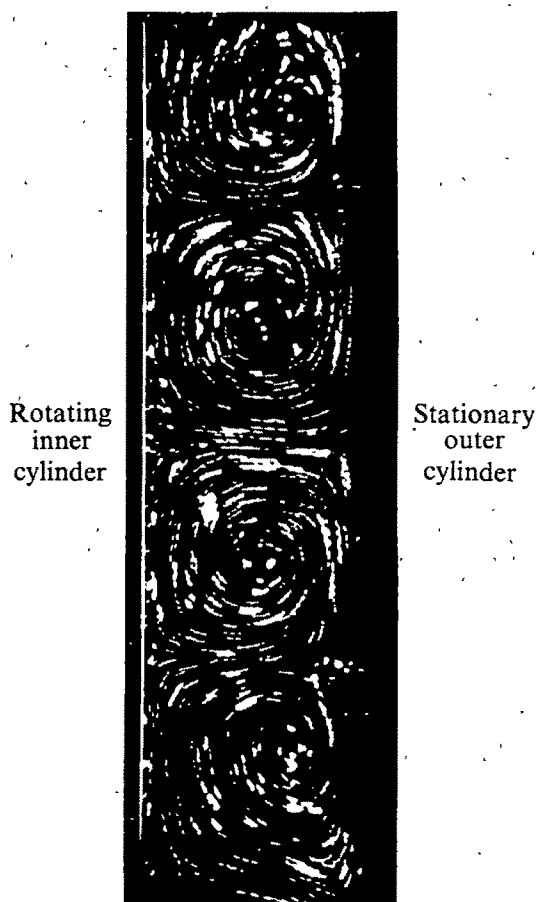
if there is a change in the onset of instability with the addition of polymer; however, our results do indicate that this change if present is not large. Changes in the onset of instability have been examined in detail by others⁵⁻⁸, however, these studies were confined to rates of rotation close to the critical region (a in Fig. 1). The large drag reducing effects we are concerned with, occur at greater rates of rotation well removed from this critical region.

By illuminating the gap between the two cylinders with a plane beam of light directed vertically along the radius and passing through the glass outer cylinder, the nature of the Taylor vortices could be examined in profile by observing scattered light from small tracer particles put into the solution.

Streamlines of the vortices observed at right angles to the incident beam are shown in Fig. 2. No detectable difference could be found in their profile with the addition of polymer, but examination of velocities using short time exposure photographs indicated that the speed of the vortices measured in a region where the vortices meet midway in the gap between cylinders, increased by up to 10% at $\omega = 13 \text{ s}^{-1}$ with the addition of polymer. This result is contrary to the expectations of some theories⁹ of drag reduction and indicates that drag reduction in Taylor vortices does not occur by suppression of vorticity; indeed the horizontal components of vorticity as represented by the secondary flow pattern of our experiments is enhanced.

We have shown that addition of polymer does not change significantly the onset of the secondary flow or suppress its vorticity and thus other mechanisms must be explored to explain the origin of the effect. We believe chain extension occurs near the cylinder walls in regions of compression where the vortex flow approaches either inner or outer wall. The possibility of

Fig. 2 Photographic profile view of Taylor vortex pattern generated in Couette apparatus; inner cylinder diameter 4 cm and gap width 1.0 cm.



chain extension occurring in the regions of extension where the vortices move away from the walls is not thought likely because related experiments firing a jet of polyethylene oxide solution at right angles to a flat surface showed localised birefringence corresponding to chain extension, near and parallel to the wall for jet outflow (axial compression) but no visible birefringence for the reverse flow direction when fluid was sucked into the jet (axial extension). We therefore conclude that in regions of compression in which chain extension occurs the extensional viscosity can be expected to increase significantly. As no drag reducing mechanism can be seen in the vertical plane we conclude that either the primary or secondary flow must be modified in the horizontal plane in such a way as to cause the reduction in overall momentum transfer to the outer wall. Further velocity profile and additional birefringence experiments viewing the horizontal plane of the Taylor vortex flow system will be carried out to establish this point.

At this stage certain generalisations concerning conditions for drag reduction may be proposed: an elongational flow system is required to produce chain extension; and the chain extension thus achieved should then be capable of reducing the momentum transfer (which is the cause of the drag) in a manner not specified in detail at the moment. This reduction of momentum transfer, however should not eliminate the elongational flow field which has produced the chain extension in the first place; its continued survival is a prerequisite for the whole effect.

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Acoustic Bragg diffraction from human tissues

ANALYTICAL methods based on the use of ultrasonic beams are potentially of interest for the remote, *in vivo* characterisation of the mechanical structure of human tissues^{1,2}. We have investigated, from this point of view, the relationship, for a specific volume of tissue, between the recorded ultrasonic backscattering amplitude and the orientation of the tissue structure to the ultrasonic beam. Experimentally this measurement is closely analogous to Bragg's X-ray crystallography arrangement except that we use 180° backscattering geometry.

Our early observations suggested qualitatively that the acoustic Bragg traces obtained were indeed characteristic of the particular tissues examined³ and modelling calculations have shown that the data are consistent with the hypothesis⁴ that diffraction arises predominantly from the connective tissue microstructure of the organs involved, for example the lobular network in liver tissue⁵. Here we present quantitative evidence that the method can provide objective characterisation of tissue structure.

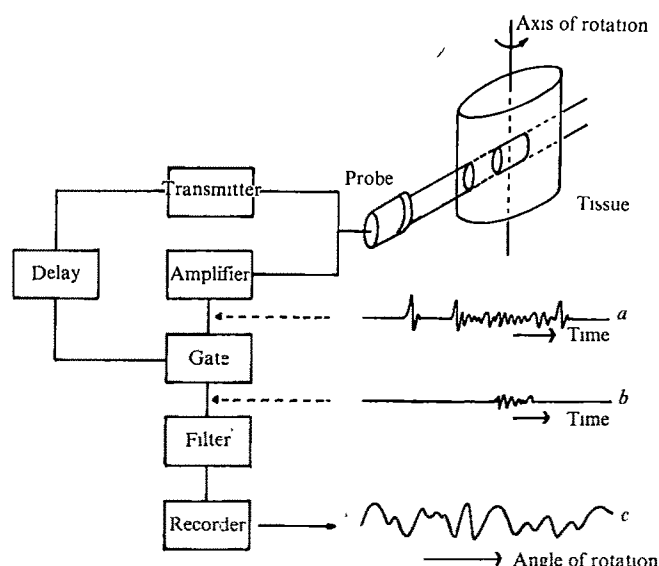
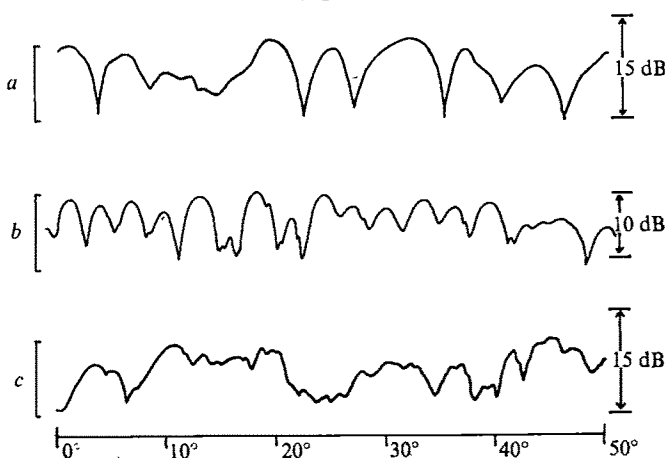


Fig. 1 Schematic diagram of the experimental arrangement for investigation of the backscattered Bragg diffraction characteristics of human tissues: *a*, received echo train from tissue specimens; *b*, gated output of received echo train; *c*, diffraction pattern obtained by frequency filtering the gated output while rotating the specimen.

Experimentally the apparatus is that of a conventional ultrasonic pulse-echo system (Fig. 1) in which an approximately cylindrical tissue specimen is positioned in a water-filled sound tank so as to lie directly in the sound beam, its long axis being normal to the direction of the beam. The sample is then rotated about this axis to enable backscattering amplitude measurements to be made from all angles. A time gate centred on this axis of rotation selects a portion of the returning echo train such that the total received echo amplitude corresponds to the backscattering from a specific volume of tissue, determined by the beam width, pulse length and appropriate time gate. Following frequency filtering of the returned echo train, plots of echo amplitude as a function of angle of rotation are obtained for one complete rotation. Some limited examples (0-50°) are shown in Fig. 2. An interesting feature of these diffraction patterns is that they seem to be characteristic of the particular tissue and presumably reflect corresponding differences between the respective tissue structures.

Evidently, several possible analytical approaches could be taken towards establishing the significance of differences or similarities between such traces and we report here the results of one of these, in which we have computed, by the fast Fourier transform method, the distributions of angular frequency

Fig. 2 Observed Bragg diffraction patterns from three types of human soft tissue, examined at 1.0 MHz. *a*, Liver; *b*, brain; *c*, spleen.



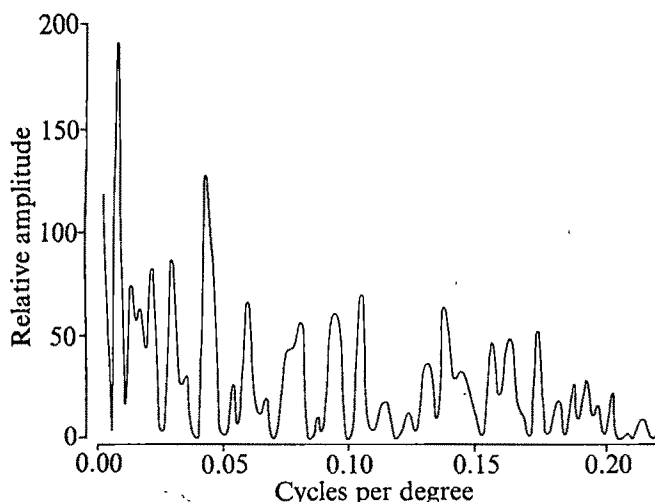


Fig. 3 Power spectra derived from the Bragg diffraction record of a specimen of human liver tissue, examined at 1.0 MHz.

components contained in the traces. A typical power spectrum obtained by this method from a single 'acoustic Bragg trace' has a somewhat complex structure, part of which may correspond to the 'picket-fence effect'⁶ inherent in the fast Fourier transform procedure (Fig. 3). For the purpose of investigating possible systematic differences between data from different tissues we have, therefore, smoothed the raw data in each spectrum by taking a seven-point average. Means of the smoothed power spectra have then been computed for each of the three sets of tissue specimens and these are plotted, together with their corresponding standard errors, in Figure 4.

These results demonstrate that this diffraction phenomenon observed in our initial investigations can be quantitatively related to the specific structure of the type of tissue. Although this finding is reasonable in view of the well known differences in the microscopic appearances of tissues of different types, its complete understanding in terms of the mechanical structure, including factors such as the existence of degrees of short range structural order, must be a subject for further research. Meanwhile, it suggests the prospect of a non-invasive diagnostic technique for distinguishing between different tissue structures *in vivo*, and thus depicting any pathological changes within a tissue region of diagnostic concern. An instrument designed to carry out such an *in vivo* investigation in the human liver has recently been constructed and will be described elsewhere.

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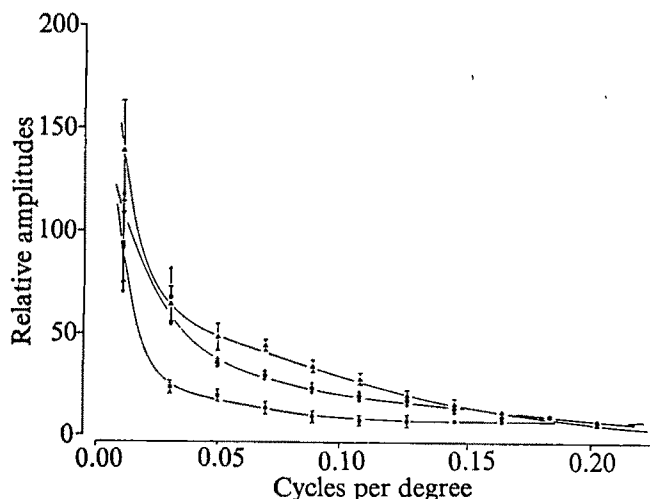


Fig. 4 Mean power spectra from three types of human soft tissue, each derived from the smoothed power spectra of twelve similar tissues examined at 1.0 MHz (▲, liver; ●, spleen; ■, brain).

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Effect on mortality of the 1974 fuel crisis

THE 1974 fuel crisis was a natural experiment. It presented the opportunity to test the hypothesis that a decrease in vehicular exhaust fumes would have a beneficial effect on health. During the first quarter of 1974 retail gasoline sales were reduced by 9.5% in San Francisco and Alameda Counties, California. Total exhaust emissions were reduced by an even greater amount because of hoarding of fuel and lowered highway speed limits. To assess the possible effects of this selective decrease in pollution, mortality rates from these two counties were examined. The rates for the first quarter of 1974 were compared with corresponding rates from the first quarters of 1970-73.

Table 1 First-quarter mortality rates per 100,000 population in two California counties, 1970-74

	San Francisco County		
	1970-73	1974	% Decrease
All causes*	377.5	327.0	13.4
Cardiovascular disease†	128.8	107.3	16.7
Chronic lung disease‡	7.3	4.9	32.9
	Alameda County		
	1970-73	1974	% Decrease
All causes*	227.1	209.7	7.7
Cardiovascular disease†	84.2	74.8	11.2
Chronic lung disease	5.0	3.1	38.0

* Excluding all deaths from vehicular accidents

† For reasons of data availability, this category includes acute myocardial infarction and chronic ischaemic and arteriosclerotic heart disease in Alameda County and all cardiac deaths in San Francisco County

‡ Includes chronic bronchitis, asthma and emphysema.

Dramatic decreases were noted in death rates for several major categories of disease (Table 1). These included all causes combined (with vehicular accident deaths excluded); pooled cardiovascular deaths; and deaths from asthma, chronic bronchitis and emphysema (denoted as "chronic lung disease"). Rates for the comparison years have been combined for simplicity of presentation. It should be noted, however, that the rates for those years were rather homogeneous. For the disease categories studied, the 1974 first-quarter rates were lower than any of the 1970-73 first-quarter rates. The 13.4% decrease in all-cause mortality in San Francisco, the more urbanised county, was almost twice that in Alameda County (7.7%). The disease category showing the greatest relative change was chronic lung disease.

In general, when the data were analysed by age, the greatest change occurred in the under 45 age group, and the least change among those over 65. We believe that this may be a result of differential exposure. Both sexes showed decreased mortality.

There was no first-quarter secular trend during the period

1970–73 for those disease categories examined. Consideration of the time trend for all quarters in San Francisco revealed a relatively repetitious and predictable seasonal variation with sharp upward swings in winter quarter death rates. During the period January 1, 1969–March 31, 1974, this cyclic trend was interrupted only by the sharp decline in mortality during the first quarter of 1974. Analysis of weather and air stability data, relevant pollutant levels and the pattern of influenza and pneumonia deaths (sometimes thought to influence cardio-respiratory deaths) support the hypothesis that a decrease in vehicular exhaust fumes would have a beneficial effect on health.

It is important that these data be analysed further and in more detail. Such analyses will include comparison of mortality rates in urban–rural subdivisions of Alameda County, consideration of the seasonal and secular trend in Alameda County for the period 1970–74, examination of several other causes of death (particularly those causes not expected to be affected by air pollution) as well as analysis of mortality data in the period following the crisis.

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Sweeping flight and soaring by albatrosses

ALBATROSSES can fly over the sea for a long time without flapping their wings. Raleigh¹ worked out a suitable method, known as dynamic soaring, to account for this and Idrac² showed that this was the method used by the birds.

Dynamic soaring makes use of the gradient in wind velocity near the surface of the sea. The bird gains energy by climbing into the wind: as it climbs it loses forward speed, but it gains airspeed by rising into the faster winds at the higher levels. It can continue to climb for as long as the wind velocity gradient is sufficient. Similarly, the bird can gain energy by losing height downwind. Combining these manoeuvres it is able to maintain flight, climbing upwind and gliding downwind in a series of swoops³.

I have suggested⁴ another method which could be used by albatrosses either in combination with dynamic soaring or as an alternative to it. Here I present calculations and a computer simulation showing that the method can provide enough energy for sustained flight.

Albatrosses often fly along the flanks of waves. This was observed by Idrac², who did not attribute any importance to it. He remarked elsewhere that he had found rising air currents directly related to the waves, in some cases greater than 2 m s^{-1} at 8 m above the sea and still detectable at 15 m. He did not think that the albatross made any use of these variations in the airflow except in exceptional cases, but they would be available for the method of soaring proposed here. It has been proposed that the birds do make incidental use of these updrafts. Pennycuik⁵ writes that "the birds use 'static' soaring along waves combined with dynamic soaring in a rather intricate way". Jameson⁷ says more specifically that the birds may use "the strong updrafts near the crests of the wave to gain considerable height—an initial kick-off, as it were, of 10 or 15 feet . . . the bird making only incidental use of the rising air currents close above the waves . . ."

The method which I propose is this static soaring to provide the initial 'kick-off', but by specifying the method more accurately it can be seen that it is more powerful than had been suspected.

The bird would take off from the crest of a wave and position itself in the rising current against the flank of the wave, maintaining this position in gliding flight. In still air, it would lose height, but in air which is rising over the wave it could maintain height by slope-soaring or could even gain height. Alternatively, it could use the upcurrent to gain speed. It could sweep along the flank of the wave, staying in the strongest upcurrents close to the surface. When the speed is sufficient, or when the bird reaches the end of the wave, it could use the speed by soaring upwards. It could then glide freely in any direction, losing height gradually until it again become necessary to swoop down and fly against the flank of a wave. Thus flight could be maintained indefinitely. This method of soaring, I shall call sweeping flight.

The height to which a bird can zoom out of slope lift can be calculated if the flight polar of the bird and the strength of the lift are known. The flight polar expresses the relationship between the flying speed V of the bird and its sinking speed S in still air. Usually, as flying speed increases the sinking speed will increase at an accelerated rate. A relatively flat polar would express the ability of the bird to fly at high speeds while maintaining a low rate of sink.

Height can be maintained by flying in air which is rising at a rate to match the sinking speed of the bird. If the bird flies close to the water in slope lift (air rising over a wave), it can increase its flying speed until its sinking speed matches the upward velocity of the air. Thus for any given upflow we can read off directly from the polar the maximum speed V_2 which the bird can reach without losing height.

Flying speed can be converted into height by climbing rapidly. If the bird can remain under control down to some minimum gliding speed V_1 , then its excess kinetic energy is given by $E = \frac{1}{2}m(V_2^2 - V_1^2)$, where m is the body of the bird. Neglecting drag, which is relatively small, the whole of this kinetic energy can be converted to potential energy by soaring to a height h . Thus:

$$h = E/mg = (1/2g)(V_2^2 - V_1^2)$$

A greater height can be reached in a wind gradient by climbing against the wind and so making use of dynamic soaring, but I shall now neglect dynamic soaring and consider only the height which can be reached by sweeping flight alone.

A bird with a flat polar will be able to reach a high speed in a relatively light upcurrent, and will be able to climb to a greater height. Birds with a flat polar will be well suited aerodynamically to the method of flight described here, whereas those with a steep polar will not be able to use it at all. This requirement, of a flat polar, is the same as that for dynamic soaring, so that a bird which is suited to one method would be suited to the other and the two methods could easily be used in combination or alternatively. Dynamic soaring could be used when the wind gradient extends up to a suitable height, perhaps 20 m; sweeping flight could be used when winds are strong but the wind gradient is not suitable.

Albatrosses, with their long narrow wings, must have a relatively flat polar, but accurate data are difficult to obtain. Bramwell and Whitfield⁸ give a curve derived from scanty data by Schmitz¹²; this was the polar used in my computer simulation. Wood⁹, for his computer simulation of dynamic soaring, used a standard aerodynamic formula with constants derived from Lanchester's¹⁰ figures for weight and wing measurements of an immature specimen of *Diomedea exulans*. This formula gives a polar closely parallel to that of Bramwell and Whitfield, but with about 0.2 m s^{-1} less sink. Thus, using the Bramwell and Whitfield polar gives a conservative estimate of performance on sweeping flight, as compared with Wood's simulation of dynamic soaring.

For waves of steepness $1/12$ and a wind of 6 m s^{-1} , the upcurrent near the wave is 1.65 m s^{-1} . This is not unrealistic, since Idrac observed even greater upcurrents in association with waves. On the Bramwell and Whitfield polar, 1.65 m s^{-1}

of sink corresponds to a flying speed of 23 m s^{-1} , and it seems that the bird can still fly at speeds of about 12 m s^{-1} . Thus, $h = 19.7 \text{ m}$. This is considerably greater than the 10 or 15 feet suggested by Jameson, and indicates that the method would be a practical one.

To obtain a more realistic estimate of performance, a program was written to simulate the sweep method of soaring on a computer. It was assumed that the wind velocity does not vary with height, and that the flow of air follows the (water) waves at the surface, so producing a sinusoidal vertical component of wind velocity; this component reduces vertically with height, reaching zero at twice the wave height. Performance is monitored by showing wave and bird on a display screen, and the print out shows flying speeds, height reached, cycle period and so on.

The simulation was run for various wind velocities and the results are shown in Fig. 1. Wave height was kept constant at 3 m. (Wave height is relatively unimportant, except in respect of giving space to manoeuvre, since wavelength tends to vary with wave height so leaving wave slope invariant.)

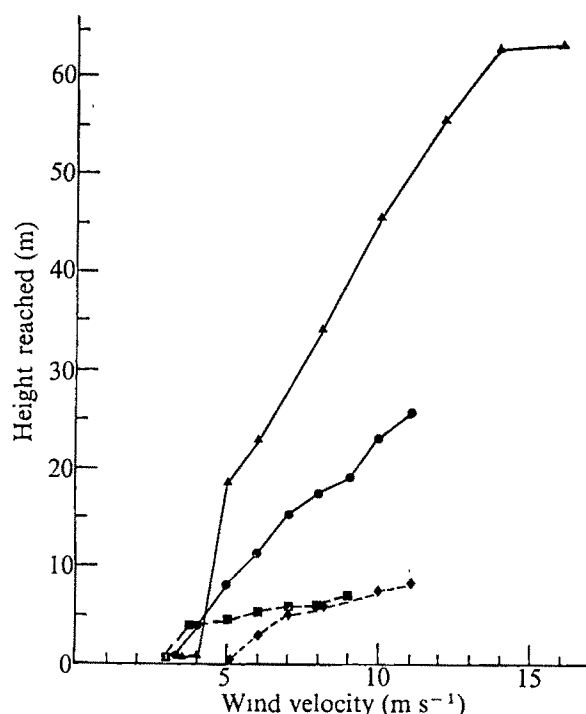


Fig. 1 Performance of four gliders using the method of sweeping flight. ●, Albatross; ■, *Pteranodon*; ♦, fulmar petrel; ▲, glider Ka6CR.

At low wind velocities ($< 3.2 \text{ m s}^{-1}$), the upcurrents are insufficient to sustain even minimum-sink gliding flight during the sweep. The bird stalls and the flight is terminated. With slightly higher wind velocities the sweep can be maintained but flying speed is increased only slightly, since the increased sinking speed of the bird soon matches the upward velocity of the air. In such cases the simulation gives short sweeps punctuated by small hops. At still higher wind velocities, the flying speed can be increased substantially during the sweep and the bird then climbs to a useful height, comparable with heights observed in practice. Maximum height reached is 26 m, with a maximum period of 62 s.

The simulation was also run with polars for the fulmar petrel, the Cretaceous flying reptile *Pteranodon* and a glider. The glider needs far more wind than the albatross to remain airborne, but far outperforms the bird at high wind velocities. The calculation is unrealistic, however, since it ignores the difficulties of placing a large glider between the waves.

The polar for the fulmar petrel *Fulmaris glacialis* was obtained by Pennycuik from measurements using a cine-camera

technique⁵. Though neither the albatross data nor those for *F. glacialis* are claimed to be accurate, it is clear that the fulmar has a greater sink at all speeds of which it is capable, and its polar is much steeper than that of the albatross. The simulation shows the fulmar reaches a maximum height of only 8 m.

The polar for *Pteranodon* was obtained by Bramwell and Whitfield from wind-tunnel measurements of a model⁶. Again, the figures cannot be claimed to be accurate, but provide a working estimate. With this polar, the simulation shows *Pteranodon* as reaching a maximum height of 7 m.

Pennycuik concluded for *F. glacialis*, and Bramwell and Whitfield for *Pteranodon*, that they were primarily slope-soarers and were unsuited to dynamic soaring. They are also far less suited to the suggested method of sweeping flight than is the albatross.

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Localisation of human peptidase-A structural locus from studies on a cultured lymphoblastoid line

LONG term genetic analysis of lymphoblastoid cell lines provides a practical means of studying the types of spontaneous and induced mutation that can occur in human somatic cells^{1,2} and of following the evolution of distinct populations of cells within a bulk culture^{3,4}. Occasionally, data may be expected to emerge which facilitate the localisation of human structural genes. We now report the first such application of this technique.

The human lymphoblastoid cell line F137 (ref. 5) has been maintained in Edinburgh since 1969 and more than 200 clones have been grown (sometimes after treatment of the bulk culture with mutagen) from single cells plated out in individual wells of plastic culture plates^{1,4}. The parent line has been examined at intervals for the electrophoretic pattern of more than 30 isoenzymes and each clone, when it has reached a sufficient cell number (usually within 2 months of isolation) has been analysed in the same way¹. Chromosome preparations have been made from both parent line and clones and analysed after staining with quinacrine dihydrochloride⁶⁻⁸.

The original F137 line was heterozygous at the pep-A locus¹ with the phenotype pep-A-2-1. In an early series of experiments (May 1971), one clone, A56 (ref. 1), seemed to be hemizygous for pep-A-2. Over the next three years, a further 133 clones were isolated from the original line; 14 had the pep-A-2 phenotype, two had an asymmetrical electrophoretic pattern with partial loss of the pep-A-1 band, and the remainder had the original symmetrical pep-A-2-1 pattern⁴. Quantitative assay of peptidase-A confirmed that loss of the pep-A-1 electrophoretic band was associated with a 50% reduction in enzyme activity⁴.

Human lymphoblastoid cell lines are not entirely stable with respect to chromosome constitution, and various rearrangements can be detected when cultures are re-examined at intervals over a period of years^{1,3}. The modal karyotype of the F137 line during the period of this study was 47, XY, the extra chromo-

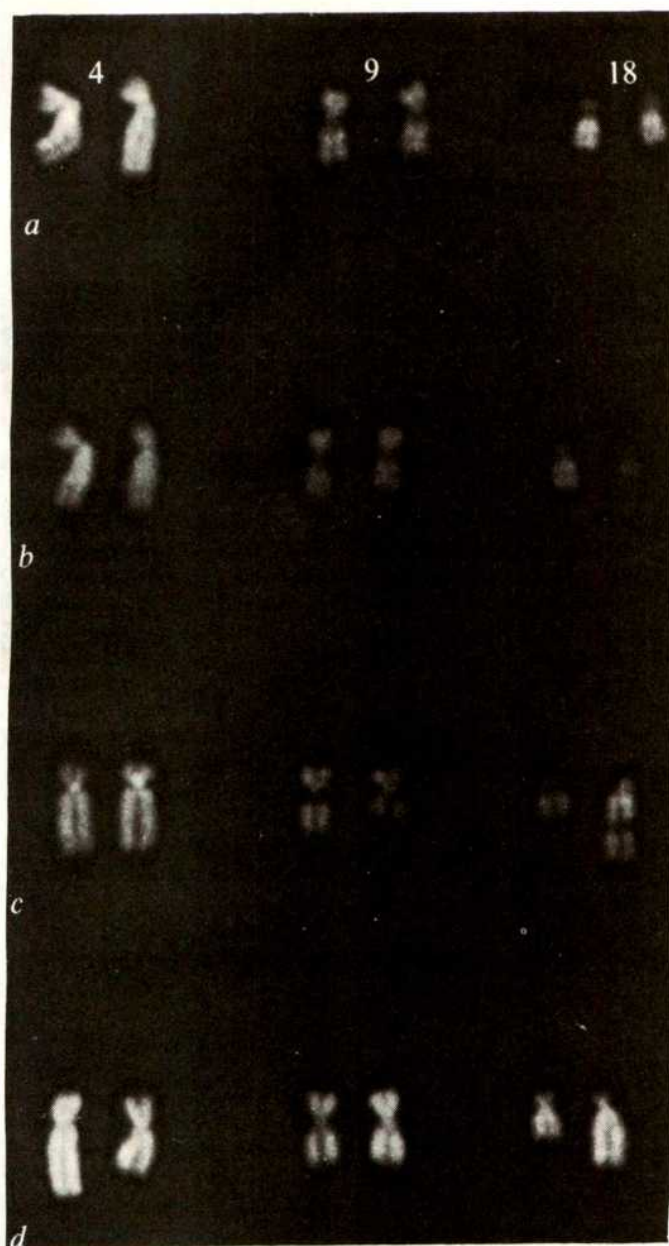


Fig. 1 Chromosome pairs 4, 9 and 18 from quinacrine-stained metaphase spreads representative of the parent line F137 (*a*), clone H₅ (*b*), clones with a 9/18 translocation (*c*) and those with a 4/18 translocation (*d*). Clones with the aberrations shown in *b* and *c* have lost activity of the pep-A-1 allele. The 4/18 translocation (*d*) is not associated with any change in pep-A phenotype.

some being a small submetacentric marker about the size of a chromosome 17/18 but distinguishable from them on quinacrine fluorescence.

The original pep-A-2 clone (A56; ref. 1) was lost before it could be examined cytogenetically. Chromosome analysis has been carried out on all subsequent clones showing alterations in the expression of the pep-A gene and on 84 of the 116 clones isolated over the same period which have retained the symmetrical pep-A-2-1 phenotype. From 4–30 metaphase spreads have been photographed and fully analysed from each clone.

Table 1 and Fig. 1 demonstrate the complete relationship between certain chromosome rearrangements involving chromosome 18 and changes in the pep-A phenotype of individual clones. One pep-A-2 clone (H₅) differed from the modal karyotype of the parent line only in having a deletion at the mid-point of the long arm of one chromosome 18 (Fig. 1*b*), a finding which supports the previous assignment of the human pep-A

Table 1 Pep-A phenotype and aberrations involving chromosome 18 in one hundred clones of F137*

Pep-A phenotype	Aberrations involving chromosome 18			
	No abnormality	4/18 translocation	9/18 translocation	18q-18q-
2-1	59	25	0	0
2	0	0	15†	1

* One isolate with an asymmetrical pep-A-2-1 pattern is excluded as not being a true clone but a mixture of cells with a 9/18 translocation and those with the F137 modal karyotype.

† Includes clone J28 with 10% pep-A-1 activity.

structural locus to the distal half of the long arm of chromosome 18 (refs 9–12).

The other 13 pep-A-2 clones all had an identical translocation (Fig. 1*c*) in which almost the whole of the long arm of one chromosome 9 was fused with the long arm of an 18, the point of fusion being marked by an intercalary band of heterochromatin from the centromere region of the 9 (Figs 1*c* and 3).

None of the 84 pep-A-2-1 clones examined contained any cells with the 9/18 translocation. Thirty-one had a karyotype identical to that of the modal cells of the parent line and 38 showed minor variations, most of which had been recognised in the parent line⁴. A further 25 had the rearrangement shown in Fig. 1*d* (cells of this type were also represented in the parent line in February/March 1973). In this case there was a break at the distal end of the long arm of a number 18 with a translocation on to it of approximately half the long arm of a number 4.

As Fig. 2 demonstrates, the break points on the long arm of chromosome 18 are indistinguishable in the 9/18 and the 4/18 translocations described above, yet in one case activity of the pep-A gene is lost, whereas in the other it is retained. Attention is therefore directed to the possible role of the translocated centromeric heterochromatin from chromosome 9.

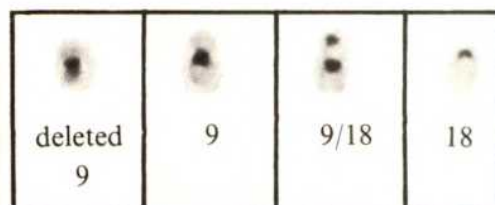


Fig. 2 Diagrammatic representation of the rearrangements involving chromosome 18 in the series of clones described in text. The break point on chromosome 18 involves the q23 region in both cases. The origin of the long arm material distal to the centromeric heterochromatin on the deleted chromosome 9 is uncertain.

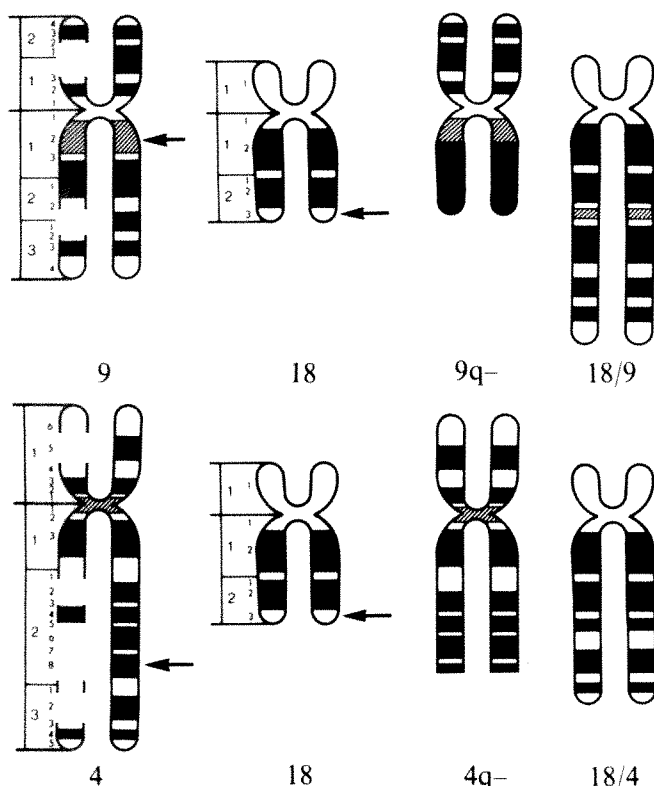


Fig. 3 C-banded preparation¹⁸ of cell from a pep-A-2 clone. The intercalary heterochromatin on the 18/9-derived chromosome is readily seen. There is some reduction in the amount of centromeric heterochromatin on the deleted chromosome 9.

In *Drosophila*, inactivation of a structural locus as a result of autosome-autosome translocation has been attributed to the suppressive effect of adjacent heterochromatin^{13,14}. (Similar gene suppression occurs when material from an autosome is translocated on to the inactive X chromosome in the mouse¹⁵.) It is usually varied in its incidence in individual cells, resulting in a variegated phenotype; further evidence that the locus in question is inactivated rather than deleted is provided if there is frequent reversion to give mosaicism or if reversions occur after recombination in which the affected locus and the adjacent heterochromatin become separated¹⁶.

One of the clones included in this study (J28) showed a very weak band in the pep-A-1 region, corresponding to approximately 10% of the normal pep-A-1 activity. On chromosome analysis every one of 30 cells examined had the 9/18 translocation shown in Fig. 1c. It is possible that this was an example of partial reactivation of the suppressed locus, although contamination with a few cells lacking the 9/18 translocation has not been completely ruled out. A second sample of this clone examined 1 month after recovery from storage in liquid nitrogen showed no detectable pep-A-1 activity.

Further studies are in progress with the aim of detecting revertants, with or without further chromosome change, in this and other pep-A-2 clones. If found, they will establish that a 'position effect' on human gene expression has been demonstrated for the first time.

Two other possible explanations for the present findings must be considered. First, since the distal fragment of the broken 18 is too small to be identified, it is possible that there has been reciprocal translocation, with retention of this fragment, in the 4/18 rearrangement but not in cells with the 9/18 translocation. Second, the break points of the 18 long arm may not be identical, being proximal to the pep-A-locus in one case but distal to it in the other. All three interpretations place the human pep-A structural locus, with some precision, in the q23 region of chromosome 18 (Fig. 2).

By virtue of their indefinite lifespan *in vitro* and their re-

lative genetic (including cytogenetic) stability, cultures of human lymphoblastoid cell lines offer considerable scope for further mapping of the human karyotype, particularly if data can be accumulated centrally from a number of laboratories over a prolonged period¹⁷.

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Regulation of chromosome 21-directed anti-viral gene(s) as a consequence of age

WHEN human fibroblast cells are cultured *in vitro* they undergo about 50 cell population doublings at which time their growth rate begins to slow down and they finally die^{1,2}. For this reason, several investigators have used the senescence of human fibroblasts *in vitro* as a model system for experimental ageing research³⁻⁶. One explanation for human fibroblast senescence is that some cell functions are lost before cells reach their maximum division limit. We have described the existence of a complex regulatory gene function controlling the inducibility of chromosome 21-directed anti-viral gene(s) (AVG) in human fibroblasts⁷. Our conclusions were derived from experiments on human fibroblasts monosomic, disomic or trisomic for chromosome 21. We therefore used all three cell types in this study to test the preservation of this complex regulatory gene function in fibroblasts allowed to age *in vitro* and in fibroblasts derived from human donors of different ages. We found (1) that no differences exist in AVG expression in human fibroblasts allowed to age *in vitro* and (2) that the AVG in human fibroblasts derived from older human donors (64 yr) is easier to induce than it is in younger donors (0-29 yr).

Human fibroblasts trisomic (T-21), disomic (D-21) and monosomic (M-21) for chromosome 21 were cultured in 75-cm² Falcon flasks and maintained with Eagle's medium containing 10% heat inactivated foetal calf serum and 1% glutamine. The cultured cells were split at a 1:4 ratio every week for 8 months. Each split is designated as a passage number so, for example, cells split during week 15 are referred to as passage 15 cells.

Table 1 Inducibility of anti-viral gene(s) in human skin fibroblasts at different cell passage

Age of donor (yr)	Cell type	No. of cell population doublings	Units HuIF required to inhibit viral RNA synthesis by 50%
3	Monosomic 21	22	0.25
		32	0.24
		58*	0.20
		64*	0.24
		66†	0.22
1		18	0.22
		64*	0.20
5	Disomic 21	28	0.062, 0.059
		32	0.064
		40	0.059
		64	0.062
		76*	0.059
		78†	0.050
2	Trisomic 21	18	0.015
		22	0.016
		66*	0.015, 0.018
		68†	0.017

In those cases where more than one value is given, the additional values represent experiments done on different days.

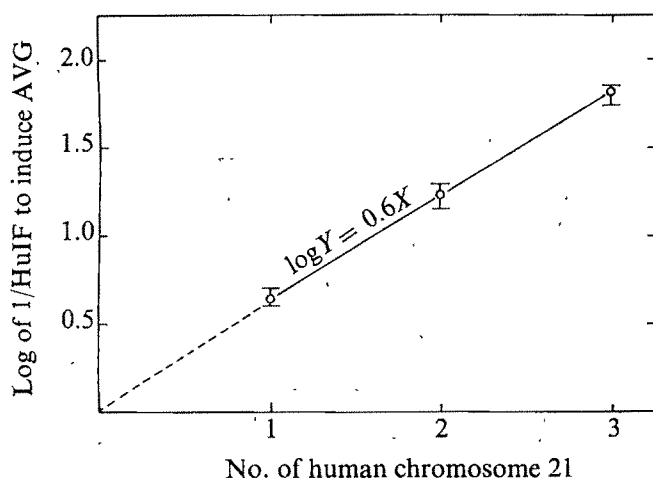
* Cell growth in phase III.

† No cell growth.

Each passage number is equivalent to two cell population doublings (passage 15 cells would have undergone 30 cell population doublings). The cells from one to two confluent flasks were frozen at each passage and stored in liquid nitrogen. The frozen cells were subsequently recultured and induced with human interferon. Concurrently the rates of cell growth were monitored at monthly intervals to determine the onset of cellular senescence. In all cases the rate of cell growth began to decrease by the time the cells had undergone 40–60 cell population doublings.

Normal diploid fibroblasts at different cell passage numbers were induced with various concentrations of human interferon. The inducibility of the AVG in these cells was assessed by the amount of human interferon required to inhibit viral RNA synthesis by 50% (ref. 7). Table 1 shows that the inducibility of the AVG remains unchanged in normal diploid fibroblasts in spite of ageing *in vitro*. Presumably this means that the regulatory gene function(s), known to control the inducibility of the AVG, also remains the same. The existence of a regulatory gene

Fig. 1 Gene dosage effect of chromosome 21 on the inducibility of the AVG in human fibroblasts monosomic, disomic and trisomic for chromosome 21. Each point represents the mean reciprocal concentration of human interferon required to induce the AVG in these cells and are derived from the values in Table 1.



function was previously demonstrated by the dosage effect of chromosome 21 on the amount of AVG product⁷. For this reason, the dosage effect of chromosome 21 on the inducibility of AVG was retested in early and late passage human fibroblasts containing differing numbers of chromosome 21. The results in Table 1 show that the dosage effect of chromosome 21 on the inducibility of the AVG remains unchanged in early and late passage M-21, D-21 and T-21 fibroblasts. These results were also plotted semi-logarithmically and a straight line represented by the equation, $\log Y = 0.6X$ (where Y denotes inducibility of the AVG and X denotes the number of chromosome 21 present) was obtained (Fig. 1). Previously, a dosage relationship expressed by the equation $\log Y = 0.61X$ was obtained⁷. The similarity in the two determinations suggests that there is no measurable loss of inducible AVG function and no significant change in the regulation of AVG expression in fibroblasts which have become senescent *in vitro*.

In the absence of measurable differences between early and late passage human fibroblasts we decided to compare the inducibility of the AVG in fibroblasts derived from human donors of different ages. In the following experiments, fibroblasts obtained from young humans (0–29 yr) are arbitrarily designated young fibroblasts and those from old humans (64 yr) are designated old fibroblasts. These fibroblasts were induced with various concentrations of human interferon and the inducibility of the AVG in these fibroblasts is presented in Table 2. The concentration of interferon required to induce the AVG in old fibroblasts is 2–3-fold lower than that required to induce the AVG in most of the young lines tested. One exception was a fibroblast line (GRC 35) derived from a 26-yr-old male which was shown to require about the same concentration of human interferon to induce the AVG as old fibroblasts. At

Table 2 Inducibility of the anti-viral gene(s) in normal skin fibroblasts derived from humans of different ages

Identification*	Age (yr)	Units of human interferon required to inhibit viral RNA synthesis by 50%
No. 141	5	0.062, 0.066, 0.063, 0.069, 0.070
GM 38	9	0.060
GM 181	10	0.059, 0.059
GM 72	11	0.060
GM 179	13	0.050, 0.050
GM 37	18	0.060, 0.052
GM 71	19	0.060, 0.062
GM 628	26	0.060
GRC 35	26	0.030, 0.040
GM 495	29	0.060
GM 288	64	0.020, 0.025, 0.028, 0.025
GRC 20	65	0.020, 0.028, 0.031
GRC 21	65	0.015, 0.015
GRC 48	73	0.015
GRC 26	75	0.030
GRC 34	80	0.030
GM 237	82	0.022, 0.040

* The GM series were obtained from Dr A. Greene of the cell repository at Camden, New Jersey. The remaining cell lines were kindly supplied by Dr E. Schneider of this laboratory. All of the cell lines were in early to early-middle passage numbers.

present such exceptions are difficult to explain. They may represent variant forms with enhanced sensitivity to the anti-viral action of interferon. The observed differences in the inducibility of the AVG in old and young fibroblasts suggest, however, that the structural gene element(s) which codes for the putative anti-viral protein or the receptor site for interferon are not as strongly repressed in old fibroblasts as in young fibroblasts. Others have also proposed similar mechanisms to explain the differential sensitivity of old and young chick embryo fibroblasts to chick interferon^{8,10}. This raises the question as to what cellular mechanisms are responsible for the differences in the different degrees of gene repression. One possibility may be that a nonspecific "relaxation" or "disorganisation" of the human genes occurs as a consequence of

age. Alternatively there may be selective pressures during the normal life span favouring the selection of cells based on their sensitivity to the anti-viral action of interferon.

It is apparent from this study that the regulatory gene function(s) controlling the inducibility of the AVG is not significantly changed in cells as a result of *in vitro* cell age.

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Mitochondrial suppressor of a nuclear gene in *Paramecium*

THE biogenesis of mitochondria depends on two sources of genetic information: one in the nucleus and the other within the mitochondria. An important question raised by this situation is how information from the two sources becomes integrated to form a functional organelle^{1,11}. Products coded by both mitochondrial and nuclear DNA have been shown to be assembled in the mitochondrial inner membrane to form complex enzymes such as ATPase, cytochrome oxidase, cytochrome *b* and mutations in either genome can affect enzyme activity^{2,6,7,14}. Since the activity of such enzymes depends on molecular interactions between their component subunits, it is conceivable that a defect in one subunit can be, at least partly, corrected by a mutation affecting another subunit. A nuclear mutation suppressing the effect of a mutated mitochondrial gene has been studied in *Neurospora*⁸. We report here the reverse situation.

The mutation was isolated in *Paramecium tetraurelia*, according to the new nomenclature¹³ and formerly *P. aurelia*, syngen 4. It is a mitochondrial mutation that partially suppresses the effects of the nuclear mutation *cl*₁. The properties of this mutation *cl*₁, already described¹⁰ are summarised first.

*cl*₁ is a monogenic nuclear recessive mutation and is characterised by (1) slow growth—a generation time of about 9 h at 27 °C instead of 6 h for wild type; (2) thermosensitivity—

most of the mutant cells die at 36 °C whereas the wild type grows as well as at 27 °C; (3) spectral deficiency in cytochrome oxidase—the absorption peak at 608 nm is about 10 times lower than in wild type; (4) incompatibility with mitochondria of wild-type origin (*M*⁺) and compatibility with those of mutant origin (*M*^{cl}): in contrast to the association between *cl*₁/*cl*₁ nucleus and *M*^{cl} mitochondria, the association *cl*₁/*cl*₁*M*⁺ results in a very reduced growth rate (generation time 15–20 h) and severely disorganised mitochondria. Although the basis (genetic, physiological or structural) of the difference between *M*⁺ and *M*^{cl} remains to be established^{3,10}, the *M*⁺ and *M*^{cl} states of mitochondria are stable and can be used as mitochondrial markers.

From the original *cl*₁ strain, a spontaneous fast growing revertant, *cl*₁-su, was isolated: its generation time at 27 °C is nearly that of wild type (7 h), although its amount of spectroscopically detectable cytochrome oxidase is not significantly different from that of the *cl*₁ mutant and its thermosensitivity is unchanged.

To establish the nature of this revertant, it was crossed with the wild type (Fig. 1A). The analysis depends on certain favourable features of the genetics of *P. tetraurelia*. The cyto-genetic events of conjugation result in identical nuclear genomes in the F₁ clones derived from each ex-conjugant¹². Commonly, cytoplasm is not passed from mate to mate and so, the two clones arising from a pair of conjugants are like a pair of reciprocal crosses. In the cross performed here, therefore, one derives its cytoplasm from the wild type, the other from the revertant parent. The second generation is obtained from the F₁ clones by autogamy; if autogamy occurs in a clone heterozygous for a pair of nuclear alleles, 50% of homozygote cells for each allele are obtained in F₂ (ref. 12).

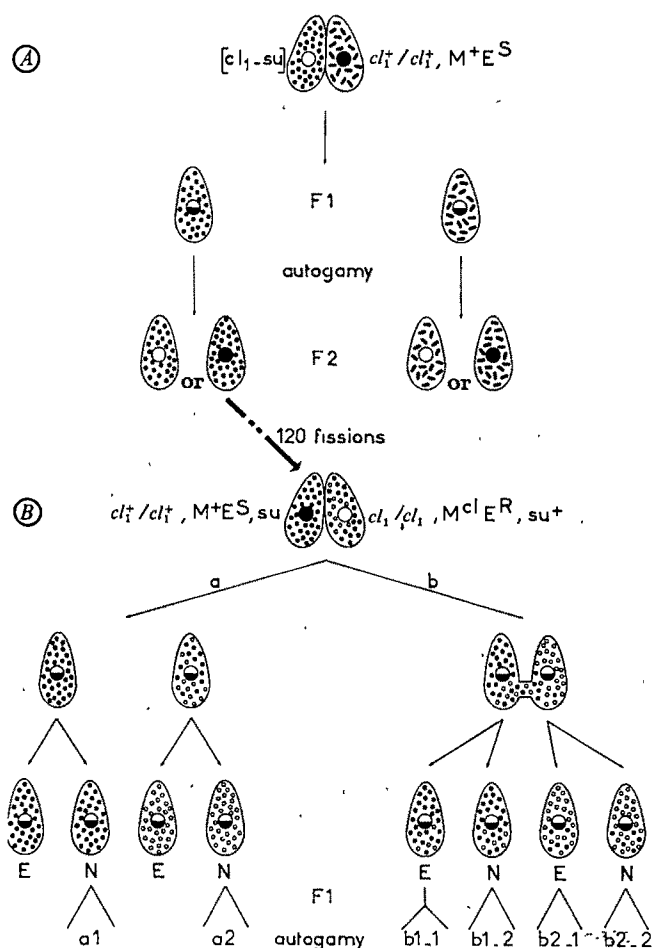
The results of the cross between the revertant (*cl*₁-su) and wild type (*cl*₁⁺), summarised in Table 1, reveal: (1) a 1:1 segregation in F₂ of the thermosensitivity property; (2) a difference between the two reciprocal crosses, ♀*cl*₁-su × ♂*cl*₁⁺ and ♀*cl*₁⁺ × ♂*cl*₁-su, as judged from the growth rate: a 1:1 segregation of slow-growing against normally growing cells appears only in the F₂ of the wild type cytoplasmic line. The phenotype of these slow growing cells clearly shows that the original *cl*₁ mutation was present and unchanged in the revertant: they have the typical generation time (15–20 h), thermosensitivity and mitochondrial alterations of the *cl*₁/*cl*₁*M*⁺ cells¹⁰. In the *cl*₁-su cytoplasmic line, the segregation of the alleles *cl*₁ and *cl*₁⁺ is only expressed by the segregation of the thermosensitivity character.

At least two hypotheses could account for these results: (1) the reversion could be due to a nuclear suppressor mutation. If so, it would have to be closely linked to *cl*₁ and would only suppress the slow growth of the *cl*₁/*cl*₁ cells when associated with *M*^{cl} mitochondria but not the interaction between gene *cl*₁ and *M*⁺ mitochondria. (2) The reversion could be due to a cytoplasmic suppressor (su). To test this second hypothesis the following experiment was carried out: the original *cl*₁/*cl*₁ strain, without suppressor, that is su⁺, was crossed with a *cl*₁⁺/*cl*₁⁺ strain derived from the *cl*₁-su cytoplasmic line of the previous cross (Fig. 1B), which was assumed to carry the

Table 1 Cross between the revertant (*cl*₁-su) and wild type (*cl*₁⁺)

Parents	Parental phenotype		F ₁ phenotype		Generation time (h)	F ₂ phenotype	No. of clones
	Generation time (h)	Thermo-resistance	Generation time (h)	Thermo-resistance		Thermo-resistance	
Revertant (<i>cl</i> ₁ -su)	7	—	6–7	+	7	+	58
					7	—	62
Wild type (<i>cl</i> ₁ ⁺)	6	+	6	+	6	+	73
					15–20	—	76

In the last column, the figures indicate the number of F₂ clones (from three different couples) studied. Average generation times at 27 °C are given. Thermoresistance or thermosensitivity at 36 °C is indicated by + or —. The cytoplasmic origin of each exconjugant F₁ clone was determined by its mating type, which is cytoplasmically inherited in this species¹².



postulated cytoplasmic suppressor, that is, su. Moreover, to test by this same cross whether the suppressor was mitochondrial or not, mitochondria from one parent (cl₁/cl₁) were marked by erythromycin resistance (M⁺E^R), those of the other (cl₁⁺/cl₁⁺) by erythromycin sensitivity (M⁺E^S). This is critical for the analysis, because it is possible in *Paramecium* to obtain matings in which cytoplasm (and mitochondria) of each mate pass to some extent into the other (Fig. 1B, b), so that each resulting clone has a mixed mitochondrial population (M⁺E^R + M⁺E^S). According to the medium (erythromycin or normal) in which these mixed clones are grown, one or the other type of mitochondria is selected^{1,9}. In this way, it is therefore possible to demonstrate whether the suppressor is associated with the mitochondria.

Two types of pairs were studied as explained in the legend of Fig. 1B; those without and those with cytoplasmic exchange between the two conjugants. Results obtained in F₂ for clones a1, a2, b1-1, b1-2, b2-1, b2-2 are given in Table 2. When no cytoplasmic exchange occurred between the two conjugants (pair a), 50% of cl₁/cl₁ cells (same generation time, 11 h, as the cl₁/cl₁M⁺E^R parent) and 50% of cl₁⁺/cl₁⁺ cells (generation time: 6 h) were obtained from the ex-cl₁ conjugant (a₂); all of these cells were E^R. In contrast, no segregation was apparent from the ex-cl₁⁺ conjugant (a₁): all the F₂ clones had a generation time of about 7 h and were E^S. This result means,

Fig. 1 Genetic analysis of the revertant. A, Cross between the revertant (cl₁-su) and wild type (cl₁⁺). ●, Cytoplasm of the revertant; ○, cytoplasm of wild type; ○, nucleus of the revertant; ●, nucleus of wild type; During conjugation, the reciprocal exchange of nuclei between the two parents yields two exconjugants (F₁) of identical heterozygote genotype ●, which differ only in their cytoplasm and therefore correspond to the two reciprocal crosses: ♀cl₁-su × ♂cl₁⁺ and ♀cl₁⁺ × ♂cl₁-su. Each exconjugant yields a clone and 20 generations later, autogamy can be induced in all cells of these F₁ clones. Autogamous cells then undergo a nuclear reorganisation which renders them homozygous for all their genes. If autogamy occurs in a clone heterozygous for a couple of nuclear alleles, a 1:1 ratio of the two homozygous genotypes is obtained in F₂ (ref. 12). B, Cross between a cl₁⁺/cl₁⁺ strain (●) derived from the revertant parent (●) and a cl₁/cl₁ strain (○) whose mitochondria carry a mutation for erythromycin resistance (M⁺E^R). This E^R mutation was selected from the original mutant strain which did not contain the suppressor (su⁺). From this cross, a pair (a) which had not undergone cytoplasmic exchange was studied as well as a pair (b) which had undergone a cytoplasmic bridge of 20 min. After the first post-conjugal fission in normal medium, one cell from each exconjugant was placed in erythromycin (150 γ ml⁻¹) (E), the other was maintained in normal medium (N): as a result, clones b1-1 (after a lag of 2-4 d) and b2-1 became pure for M⁺E^R mitochondria, and clones b1-2 and b2-2 for M⁺E^S mitochondria. The F₂ analysis of these clones is given in Table 2.

therefore, that the cytoplasm of the cl₁⁺ strain did carry the suppressor and maintained it over 120 cellular generations of association with a wild-type genotype (Fig. 1B). Furthermore, it can be seen that this suppressor also suppresses the incompatibility between gene cl₁ and mitochondria from the wild type, for the cl₁/cl₁M⁺su cells have a generation time of 7 h.

Information concerning the mitochondrial location of the suppressor can be derived from the study of the exconjugants which have exchanged cytoplasm (pair b). The presence of the suppressor in these exconjugant clones can be determined by the growth rate of the F₂ cl₁/cl₁ cells derived from them.

The mitochondrial composition of these clones (containing both M⁺E^R and M⁺E^S mitochondria after conjugation) will depend on the medium (E or N, Fig. 1B) in which they will be put, one fission after conjugation. In erythromycin, they will become pure for E^R mitochondria, in normal medium, pure for E^S ones^{1,9}. A very strict correlation between the presence or the absence of M⁺E^S mitochondria and the presence or the absence of the suppressor was observed (Table 2). When the F₂ progeny of a clone is E^S (b1-2), the generation time of the cl₁/cl₁ cells is identical to that of the cl₁⁺/cl₁⁺ ones and therefore the suppressor is present. When the F₂ progeny is E^R (b1-1 and b2-1), the generation time of the cl₁/cl₁ cells is 11 h: the suppressor is absent. The joint loss of E^S mitochondria and of the suppressor in clone b1 is particularly striking. Conversely, the joint gain of the E^S mitochondria and of the suppressor was observed in clone b2-2. While clones b1-1, b1-2, b2-1 had become pure for one mitochondrial type, clone b2-2 was still mixed (M⁺E^Rsu⁺, M⁺E^Ssu⁺) when autogamy occurred: this was shown by the intermediate growth rate and resistance to erythromycin of the 13 cl₁/cl₁ cells when they were first tested (Table 2). Five fissions after autogamy, cells from these 13 clones were still mixed and could become pure for either E^R mitochondria and the su⁺ condition (generation time: 11 h)

Table 2 F₂ progenies of the cross cl₁⁺/cl₁⁺ M⁺E^S su × cl₁/cl₁ M⁺E^R su⁺ after growth in erythromycin (E) or normal medium (N) of the F₁ clones

F ₁ clones	a1 (N)	a2 (N)	b1-1 (E)	b1-2 (N)	b2-1 (E)	b2-2 (N)
Genotype	cl ₁ /cl ₁	cl ₁ ⁺ /cl ₁ ⁺	cl ₁ /cl ₁	cl ₁ ⁺ /cl ₁ ⁺	cl ₁ /cl ₁	cl ₁ ⁺ /cl ₁ ⁺
Generation time (h)	7	11	11	6	11	6
Erythromycin resistance	-	+	+	-	+	+
No. of clones	85	39	14	15	30	13

The origin of the clones a1, a2, b1-1, b1-2, b2-1, b2-2 is given in Fig. 1B. The F₂ cells (cl₁/cl₁ and cl₁⁺/cl₁⁺) were tested for their resistance or sensitivity to erythromycin about three generations after autogamy and their growth rate was recorded. Average generation times at 27 °C are given. The erythromycin resistance or sensitivity is symbolised by + or -; ± indicates that the cells first were blocked in erythromycin and then progressively became resistant.

or for E^s mitochondria and the su condition (generation time: 7 h) according to the medium (E or N) in which they were grown.

A last point is worth consideration. This suppressor interacts not only with the product of the mutated gene *cl₁* but also with the product of the wild-type allele, *cl₁⁺*: the *cl₁⁺/cl₁⁺su* cells have a slightly increased generation time (7 h instead of 6) and a strongly decreased cytochrome oxidase content (A. S., unpublished).

In conclusion, I have identified in the *cl₁-su* strain a suppressor which behaves genetically as a mitochondrial mutation, since its transmission is linked to the mitochondrial E^s marker. The mechanism of this suppression remains to be established. It is probably not a direct informational suppression because it is very unlikely that mRNA transcribed in the nucleus is translated by mitochondrial tRNAs⁵. It is more likely an indirect suppression by functional interaction between two modified products, the one nuclear, the other mitochondrial. The identification of the product coded by this new mitochondrial mutation should provide information on the informational content of the mitochondrial DNA and on the problem of the interactions between products coded by the nuclear and mitochondrial genetic systems.

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Spontaneous AKR lymphoma with T and B-cell characteristics

THE existence of surface immunoglobulin on thymus-derived cells (T cells) has been difficult to demonstrate and is still controversial¹⁻⁶. Therefore the presence of easily detectable surface immunoglobulin (sIg) is considered to be a reliable marker for lymphoid cells of bone marrow origin (B cells)⁷. To our knowledge no cell line has been reported to bear both easily detectable sIg (that is, by immunofluorescent staining) and T-cell markers, although two murine T-cell lines have been reported to bear small amounts of sIg detectable only by extremely sensitive methods^(2,6). We now report the existence of a murine lymphoma line with both T and B-cell characteristics by the criteria of easily detectable sIg, presence of Thy1.1 (θ AKR) antigen⁸, and mitogen responsiveness.

The tumour line, designated AkTB-1, originated as a spontaneous lymphoma in the lymph nodes and spleen of a 14-month-old AKR/J mouse which had been thymectomised at 1 month of age. Lymph node (LN) cells were initially passaged, and the line was subsequently maintained *in vivo* by serial intravenous passage of 10⁵ spleen cells into young AKR/J male hosts. For comparison, cells from age-matched normal AKR/J mice or from AKR/J mice with spontaneous thymomas were studied.

Thy1.1 antigen was detected by specific immunofluorescent staining using an indirect technique. Spleen or LN cells were incubated with anti-Thy1.1 antiserum prepared by hyperimmunisation of CBA/J mice with AKR/J thymocytes. The

Table 1 Thy1.1 antigen determinations

Donor	No. of experiments	% Positive cells ± s.e.	
		Spleen	LN
Normal 3 month AKR	6	38 ± 4	71 ± 3
AkTB-1 in passage*	9	94 ± 1	91 ± 1
AKR spontaneous thymoma	3	98 ± 1	98 ± 1
AKR lymphoma in passage†	8	96 ± 4	92 ± 2

*Experiments performed after day 12 of passage.

†Cell lines derived from spontaneous AKR thymomas passaged as spleen cells. One hundred cells were counted in each experiment.

cells were washed and then counterstained with fluorescein-conjugated goat anti-mouse γ₂ heavy chain antibody. The reactivity of this anti-Thy1.1 antiserum against thymus cells could be removed completely by absorption with AKR/J brain tissue, and less than 2% of AKR/J spleen cells react with anti-γ₂ antiserum.

Lymph nodes and spleens were removed from mice on various days after passage and examined for surface Thy1.1 antigen (Table 1). By day 12, more than 90% of LN and spleen cells were Thy1.1 positive. This pattern was maintained throughout the passage. Similar results were obtained with cells from AKR mice bearing a spontaneous thymoma or another passaged AKR lymphoma cell line derived from a spontaneous AKR/J thymoma.

sIg was determined by Dickler's method¹⁰. Cells were incubated with fluorescein-conjugated goat or rabbit antibody monospecific for α, μ, γ₁, and γ₂ heavy chains, as well as with a fluorescein-conjugated polyvalent anti-mouse immunoglobulin serum. The percentage of sIg positive cells was determined at various times after passage (Fig. 1). AkTB-1 spleen cells progressively acquired sIg of the IgM class; whereas fewer than 10% of cells were sIg positive on day 14, more than 90% were positive by day 22. At all times, fewer than 5% of cells could be stained for α, γ₁ or γ₂ heavy chains (data not shown).

Fig. 1 Percentage surface immunoglobulin positive cells in LN and spleen of AkTB-1 bearing animals. Open circles represent spleen cells; closed circles, LN cells. sIg represents staining for μ heavy chain on cell surfaces. Upper bracket shows range for normal spleen cells. Lower bracket shows range for normal LN cells.

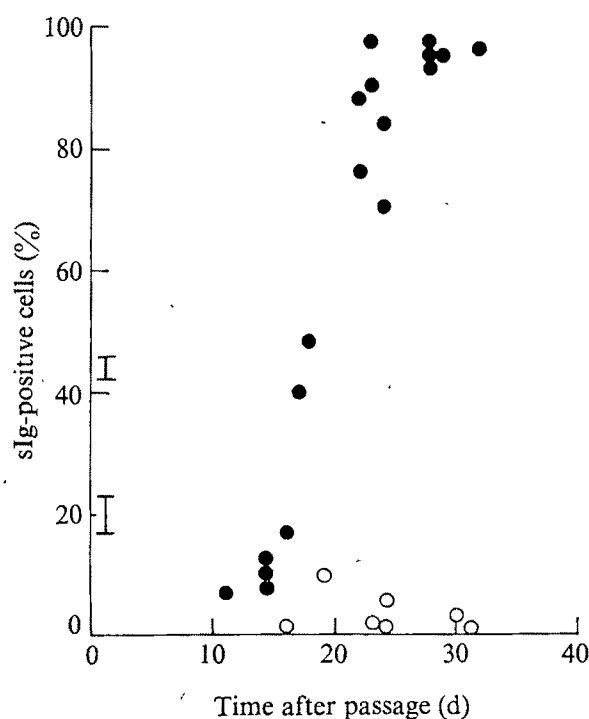


Table 2 Capping and regeneration of surface markers

	1 h after capping			24 h after capping		
	% Positive cells*	% Caps	% Rings†	% Positive cells	% Caps	% Rings
Thy1.1	100	75	25	100	0	100
sIg	74	89	11	77	16	84

*Expressed as percentage of total spleen cells.

†Expressed as percentage of positively stained cells, see text for explanation of terms. 2×10^7 spleen cells from an AkTB-1-bearing mouse late in passage were incubated with unlabelled anti-Thy1.1 antiserum for 30 min at 4 °C, washed and then incubated with unlabelled goat anti-mouse γ_2 heavy chain antibody for 1 h at 37 °C. Either at this point or after culture overnight, cells were examined for Thy1.1 antigens (see text). For detection of sIg regeneration, a single incubation at 37 °C for 1 h with unlabelled anti-mouse immunoglobulin was performed before overnight culture. Cells were stained according to method in text.

Thus almost all AkTB-1 spleen cells late in passage bear both IgM and Thy1.1 on their surface. Few LN cells from the same animals bore sIg even late in passage when lymph nodes were grossly and microscopically replaced with tumour. Neither spleen nor LN cells from three spontaneous or two other passaged AKR lymphomas had detectable (>2%) sIg of any class.

Since the coexistence of easily detectable T and B-cell markers on the same cell is unusual, we wished to test the possibility that either Thy1.1 or sIg was passively acquired. In one experiment (Table 2), Thy1.1 on the surface of AkTB-1 spleen cells was capped by incubating the cells first with unlabelled anti-Thy1.1 serum, then with unlabelled anti-mouse γ_2 heavy chain at 37 °C¹¹. Cells stained immediately after these incubations were 100% positive for Thy1.1; in 75% the antigen was present only in the form of caps, while 25% still had a circumferential (ring) distribution of Thy1.1. After overnight incubation, cells reacted with Thy1.1 antisera and counter-stained with fluorescein-conjugated anti- γ_2 antibody all exhibited a fluorescent ring pattern. Staining with only anti- γ_2 showed 92% of cells were still capped, indicating that the Thy1.1 caps formed a day earlier had neither shed nor redistributed themselves over the cell surface. These results suggest that AkTB-1 spleen cells can generate Thy1.1 antigen.

Similar results were obtained for capping and regeneration of surface immunoglobulin (IgM) on AkTB-1 spleen cells (Table

2). In addition, preliminary studies indicate that these cells synthesise IgM *in vitro*.

Mitogen-stimulated DNA synthesis by AkTB-1 cells was determined using a microculture method. Responsiveness to phytohaemagglutinin (PHA) and lipopolysaccharide (LPS) at early, middle and late points in a typical passage are shown in Table 3. At day 7 of passage, when lymphoid organs were not yet enlarged and the frequency of Thy1.1 positive spleen cells was only slightly increased, mitogen responsiveness of AkTB-1 spleen cells was comparable with that of normal cells. By day 14, when lymphoid organs were enlarged 10-fold and spleen cells were 94% Thy1.1 positive and 33% sIg positive, spontaneous DNA synthesis was elevated, but was depressed by PHA and LPS. Late in passage when AkTB-1 spleen cells were 95% Thy1.1 and 83% sIg positive, they exhibited a high spontaneous level of DNA synthesis which was depressed by PHA but markedly stimulated by LPS. Mitogen responsiveness of AkTB-1 LN cells (Table 3) was not remarkably different from that of normal AKR cells. These results further suggest the dual nature of the spleen cells, since LPS responsiveness is considered to be characteristic of B cells¹², whereas suppression of DNA synthesis in response to PHA has been reported as a characteristic of some murine T-cell lymphoma lines^{13,14}.

AKR lymphoma usually arises in the thymus, presumably as a result of viral transformation of a susceptible cell population, and can be prevented by thymectomy¹⁵. The

Table 3 Mitogen-stimulated ³H-thymidine incorporation by AkTB-1 and normal lymphocytes

	AkTB-1		Normal	
	Spleen	LN	Spleen	LN
Day 7				
Thy1.1 (%)	64	72	32	—
sIg (%)	49	16	48	—
Yield*	1×10^8	4×10^7	8×10^7	—
Medium†	829 ± 69	120 ± 11	$1,051 \pm 316$	318 ± 77
PHA	$16,651 \pm 1,251$	194 ± 6	$23,518 \pm 1,338$	$19,152 \pm 1,317$
LPS	$9,615 \pm 1,403$	128 ± 88	$15,236 \pm 661$	324 ± 74
Day 14				
Thy1.1	94	94	25	70
sIg	33	18	42	20
Yield	1×10^9	5×10^8	1×10^8	3×10^7
Medium	$29,564 \pm 887$	$2,405 \pm 382$	$1,872 \pm 329$	$2,537 \pm 988$
PHA	$10,253 \pm 408$	$5,708 \pm 324$	$9,319 \pm 1,880$	$5,231 \pm 1,229$
LPS	$8,055 \pm 978$	$1,962 \pm 848$	$5,932 \pm 853$	$2,318 \pm 495$
Day 21				
Thy1.1	95	95	32	72
sIg	83	5	38	18
Yield	1×10^9	5×10^8	9×10^7	6×10^7
Medium	$35,493 \pm 1,791$	366 ± 53	501 ± 27	305 ± 25
PHA	$17,554 \pm 1,495$	$2,451 \pm 5$	$3,994 \pm 135$	$2,177 \pm 205$
LPS	$83,733 \pm 1,508$	$1,148 \pm 175$	$5,273 \pm 250$	352 ± 5

*Yield indicates total number of nucleated cells in spleen or lymph nodes (inguinal, axillary and mesenteric).

†Data are mean c.p.m. \pm s.e. of triplicate cultures at the mitogen concentration resulting in optimal stimulation (PHA, $1.6 \mu\text{g ml}^{-1}$; LPS, $100 \mu\text{g ml}^{-1}$).

Cells were suspended at a concentration of 10^7 cells per ml in RPMI 1640—10% FCS with 2 mM fresh glutamine and antibiotics. 0.1 ml of cells was incubated in microtitre plates with an equal volume of medium or doubling dilutions of PHA or LPS. The cultures were incubated at 37 °C in 5% CO₂ in air for 18 h, then pulsed with $1.0 \mu\text{Ci } ^3\text{H}$ -thymidine for a further 6 h before collection.

origin of the AkTB-1 tumour line as a spontaneous lymphoma in a thymectomised mouse suggests that the absence of the thymus was important in the generation of the unusual double-marker characteristics of these cells. This hypothesis is supported by the recent appearance in our laboratory of a second sIg positive, Thy1.1 positive spontaneous tumour originating in another thymectomised AKR/J mouse. It may be that the absence of susceptible thymus cells allows the subsequent transformation of a normal double-marker cell population. Such a subpopulation has been described in man¹⁶⁻¹⁸. Alternatively the thymus may modulate neoplastic as well as normal cell differentiation, so that in its absence transformed cells develop atypical characteristics.

Regardless of the origin of these cells, the progressive acquisition of B-cell characteristics as well as their differential expression in spleen and lymph node suggest that these neoplastic cells undergo differentiation *in vivo* and that the lymphoid tissue microenvironment has a role in the control of this differentiation process.

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Precommitment of normal mouse peritoneal cells by erythrocyte antigens in relation to auto-antibody production

We have reported that peritoneal cells (PCs) from unstimulated mice can, after 2 h of incubation in the appropriate medium, start to secrete anti-sheep erythrocyte (SRBC) antibodies^{1,2}. We extended this observation by showing that PCs cultured in standard conditions for 4-6 d in the absence of SRBC, can form numerous haemolytic plaques, demonstrable by the ordinary techniques of local haemolysis (in Agarose, in liquid layer or in cellulose gum³). The immunological nature of this plaque-forming activity was established by the following criteria: complement dependency, inhibition by anti-mouse IgM serum and immunological specificity. Thus, we concluded^{3,4} that PCs of normal adult mice had been stimulated previously by an unknown immunogen sharing common or cross-reacting determinants with SRBC, and that tissue culture conditions would derepress the built-in capacity of these cells to produce antibodies. Treatment of autologous erythrocytes by the proteolytic enzyme bromelain revealed that normal mouse spleen cells

regularly produce antibodies against their own erythrocytes^{5,6}. This led us to investigate the behaviour of normal PCs towards SRBC or mouse red blood cells (MRBCs) treated by bromelain (BrMRBCs). We report here that mouse PCs in culture develop a plaque-forming activity against isologous BrMRBC as well as against SRBC, and we show that these two types of erythrocytes share some common antigen.

PCs from non-immunised 22-28-week-old CBA/J or NZB mice were cultured by Mishell and Dutton's technique⁷ at a concentration of 4×10^6 ml⁻¹ in RPMI 1640 supplemented with 10% foetal calf serum. Cell viability was measured by Trypan blue exclusion. The plaque-forming activity of lymphoid cells was measured by Cunningham's liquid layer technique⁸ or the carboxymethyl cellulose technique (CMC)^{4,9}. SRBCs or MRBCs were treated with bromelain as described by Cunningham⁵. For mixed plating, an equal number (5×10^6 ml⁻¹) of BrMRBCs plus SRBCs or of BrMRBCs plus BrSRBCs was used. A mixture of equal volumes of fresh rabbit and guinea pig sera was used as a source of complement, the final concentration being 10%.

PCs from normal mice produced no plaques of haemolysis against MRBC in the liquid layer technique, and only an insignificant background was detected by day 4 of culture with the CMC technique (Table 1). On the other hand, as already described³, increasing number of plaque-forming cells (PFCs) developed against SRBCs during culture. The highly sensitive CMC technique¹⁰ provided many more plaques than the liquid technique.

If mouse erythrocytes were treated with bromelain their antigenic properties were modified so that they became sensitive to antibodies secreted by mouse PCs (Table 1). In most cases treatment of SRBCs by bromelain also led to an increase of the PFCs expressed by PCs in culture.

The behaviour of spleen cells from normal mice cultured in the absence of SRBCs was similar to that of PCs: although almost no PFCs against MRBCs and only a few background plaques against SRBCs could be detected, treatment of these erythrocytes with bromelain resulted in the appearance of a substantial number of PFCs in the spleen cell population (Table 2).

The immunological nature of the plaque-forming activity against BrMRBCs and BrSRBCs was demonstrated by the fact that no plaques appeared in the absence of complement and that 95% of PFC were inhibited by a specific anti-mouse IgM serum, added to the liquid medium or CMC at a final concentration of 1%. The system displayed immunological specificity inasmuch as horse red blood cells (HRBCs) or bromelain-treated HRBCs never gave any significant number of plaques with normal peritoneal or spleen cells (SCs). Furthermore, ghosts or SRBCs or BrMRBCs, obtained as described by Dodge *et al.*¹¹, inhibited plaques against SRBCs and BrMRBCs whereas ghosts of HRBCs did not.

As both SRBCs and BrMRBCs could be lysed by cultured peritoneal and spleen cells it was tempting to speculate that the cells share common or cross-reacting determinants. To check this hypothesis, both sorts of cells were plated with a mixture of both types of erythrocyte, and the morphology of plaques of haemolysis was examined¹². Three types of plaque were observed: (1) clear plaques, (2) 'sombrosos', where the centre was clear and the periphery cloudy and (3) incomplete or cloudy plaques. Clear plaques and sombrosos were judged as indicating cross-reactivity between antigenic determinants on the surface of both types of erythrocyte present in the gel, whereas cloudy plaques were thought to be formed in the absence of such cross reactivity. As Table 3 shows, SRBCs and BrMRBCs cross reacted slightly (10%) while BrSRBC and BrMRBC cross reacted to a large extent (40%).

These results lead us to interpret the spontaneous plaque-forming activity of PCs, and at a lower level of spleen cells, as a manifestation of an autoimmune process. As Cunningham proposed¹³, young mice would be sensitised to antigenic determinants present on their own erythrocytes but hidden in the

Table 1 Development of spontaneous plaque-forming cells by CBA mouse peritoneal cells in culture

Days in culture	Plaque assay system	MRBC	Plaque-forming activity* of PCs plated on different types of erythrocytes	BrSRBC†	SRBC	BrSRBC†	HRBC	BrHRBC†
0‡	Liquid§	0	2±4	0	0	0	0	0
	CMC¶	0	3	24±5	79±15	0	0	0
4	Liquid	0	3,963±425	ND	ND	1±4	4±5	4±5
	CMC	2±4	29,592±3,899	7,169±1,472	6,918±762	5±2	4±5	4±5
	Liquid	0	5,700¶	1,313±259	3,484±951	ND	ND	ND
6	CMC	0	31,510±3,950	8,379±2,339	27,490±3,180	ND	ND	ND

* Plaques per million viable recovered cells ± s.e.

† Bromelain-treated mouse (M), sheep (S) or horse (H) red blood cells.

‡ Peritoneal cells were collected, washed and placed directly into plaque assay medium without previous culturing.

§ Carboxymethyl cellulose gel; readings after 2 h at 37 °C.

¶ Readings after 1 h at 37 °C.

¶ Approximate number; at the dilution used the number of plaques per slide was too high to be counted accurately.

Table 2 Development of spontaneous PFCs by CBA mouse spleen cells in culture

Days in culture	Plaque-forming activity† of SCs plated on different types of erythrocytes	MRBC	BrMRBC†	SRBC	BrSRBC†
0‡	3±5	176±53	11±2	340±88	
1	1±4	233±35	10±10	400±60	
2	7±8	1,273±191	5±8	268±85	
4	0	823±179	51±26	145±32	

* Plaques per million viable recovered cells ± s.e., counted after 1 h at 37 °C; liquid medium technique.

† Bromelain-treated mouse (M) or sheep (S) red blood cells.

‡ Spleen cells were collected, washed and placed directly into plaque assay medium without previous culturing.

Table 3 Formation of clear plaques, sombrero and incomplete plaques by peritoneal cells in culture when plated with a mixture of SRBC and MRBC

Indicator cells	Activity of the peritoneal cells (PPM†)			
	Clear plaques	Sombreros	Incomplete	% double plaques§
SRBC (1)	2,233±496	0	0	
(Br)SRBC* (2)	5,300±662	0	0	
MRBC (3)	0	0	0	
(Br)MRBC* (4)	11,766±3,636	0	0	
MP‡ (1)+(3)	0	0	1,360±296	0
MP‡ (1)+(4)	67±103	766±585	7,533±1,998	9.4±5.2
MP‡ (2)+(4)	1,640±712	2,400±316	6,080±1,180	40±7.9

NZB peritoneal cells were cultured for 6 d (CBA mouse PC gave comparable results). 'C' was a mixture of equal parts of guinea pig serum and rabbit serum checked for absence of haemolytic activity against the erythrocytes used.

* Bromelain-treated sheep (S) or mouse (M) red blood cells.

† Plaques per million recovered viable cells ± s.e.; readings were performed after 1 h at 37 °C, liquid medium technique.

‡ MP, mixed plating; red cells were mixed in equal proportions as indicated.

§ Number of clear and sombrero plaques as percentage of the total plaque number.

membrane. If these epitopes, unravelled on MRBC by bromelain, are also present in an exposed form on SRBCs, auto-antibody secreted by peritoneal and spleen cells would attach spontaneously on SRBCs.

A repressive mechanism seems to restrict the number of PFCs *in vivo* to a low level¹⁸. Our results show that, in culture conditions, their number increases considerably. Thus, the immune behaviour of PCs becomes a phenomenon of general biological importance, and perhaps an index of a spontaneous autoimmune process. Our findings underline the need for a reassessment of the concept of 'primary response' of mice to SRBCs.

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Noradrenaline induces morphological alterations in nucleated and enucleated rat C6 glioma cells

GILMAN and Nirenberg¹ have reported that catecholamines induce a striking increase in intracellular cyclic AMP concentration in cultures of rat and human glioma cells. They concluded that this activation of the adenylate cyclase was mediated through the β -adrenergic receptor, since the effect was inhibited by the β -receptor blockers sotalol and dichloroisoproterenol. Another report² showed that cultured human glioma cells treated with cyclic AMP derivatives resumed the typical morphology of the normal counterparts, that is, having multiple processes extending from a compact cell body. I have therefore compared the effects of noradrenaline, a β -adrenergic stimulator, on the morphology and cyclic AMP level in rat C6 glioma cells.

I have also investigated whether the variations in cell shape require nuclear activity. The results demonstrate that the morphological alteration occurs in normal cells as well as in cells enucleated by cytochalasin B. Moreover, the effect of noradrenaline on cyclic AMP concentration and cellular morphology is transient.

In the absence of noradrenaline (Sigma) the nucleates and the enucleates have an irregular flattened shape (Fig. 1a and c, respectively). Treatment of the C6 cells and the enucleates with the drug produced a marked change in morphology; the cells and the enucleates assumed a spindle-like morphology (Fig. 1b and d, respectively). The cytoplasm retracted to form a compact cell body with multiple processes. Occasionally the treated nucleates as well as enucleates had bipolar processes. Now and then the processes showed beadings and bifurcations. Routinely, I used 10^{-5} M noradrenaline; concentrations as low as 10^{-7} M are sufficient to induce the morphological change, whereas 10^{-8} M noradrenaline has no effect.

The effects of noradrenaline on the morphology of the nucleates and the enucleates was inhibited by the β -receptor blocking agent DL-propranolol (Sigma, at 10^{-5} M), but not by phentolamine (Ciba-Geigy, 10^{-6} - 10^{-4} M), a specific α -receptor blocker.

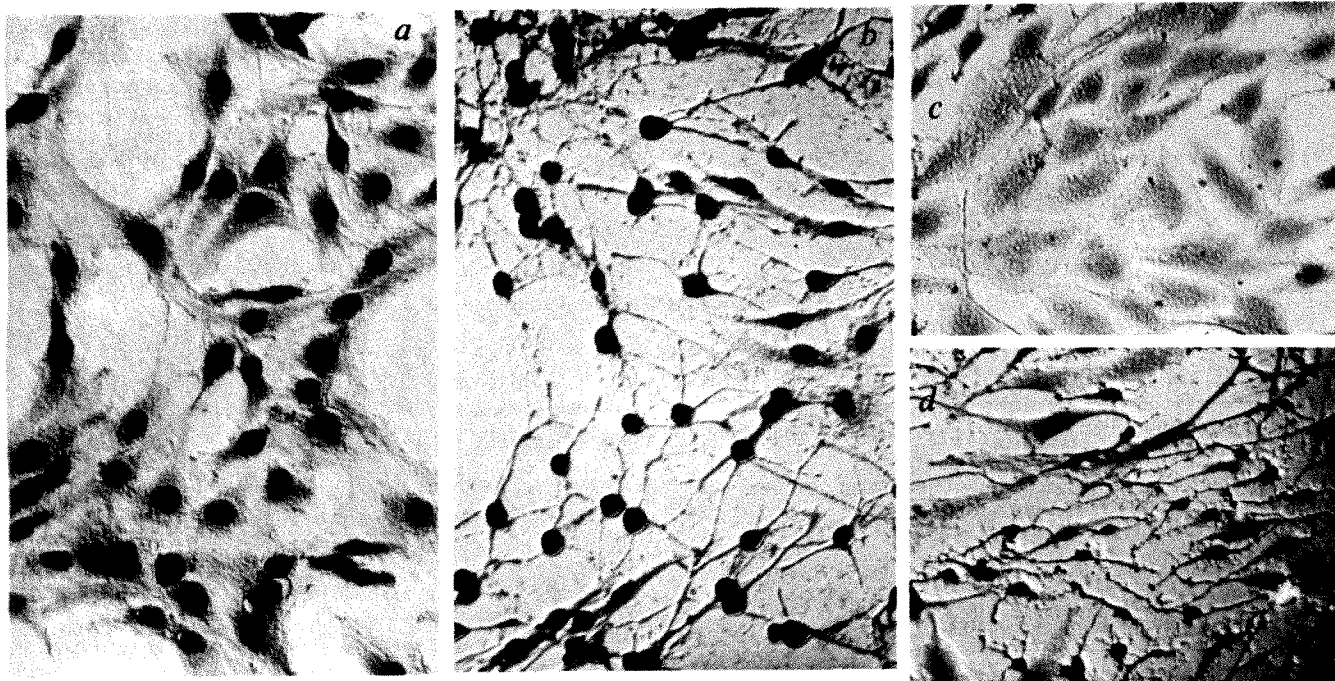


Fig. 1 Demonstration of the morphological change in nucleated and enucleated rat C6 glioma cells caused by noradrenaline. The C6 rat glioma cells used throughout the experiment were constructed by Benda *et al.*³ and were obtained from Flow Laboratories. The cells were routinely maintained in Ham's F10 medium (Flow Laboratories, contains 0.25 mM Ca^{2+}) supplemented with 15% horse serum and 2.5% foetal bovine serum in a humidified 5% CO_2 incubator at 37 °C. In the actual experiments, the cells were allowed to recover from trypsinisation for at least 48 h in fresh medium. For the enuclea-

tion of C6 cells, the protocol described by Prescott *et al.*⁴ was followed, which in the case of C6 cells yielded more than 90% enucleation. The enucleates were then placed back in the original medium at 37 °C for 30 min to allow them to assume a normal appearance before starting the experiments. Nucleates (a) and enucleates (c) in the absence of noradrenaline and in the presence of 10^{-6} M of noradrenaline for 2 h (b and d, respectively). The cells were fixed in 100% methanol and stained with Giemsa. The micrographs were taken with Zeiss interference contrast optics. Magnification $\times 224$.

The morphological change in C6 cells, nucleates and enucleates, stimulated by noradrenaline, could be inhibited by colchicine (Serva, 1 mg ml^{-1}), added together with noradrenaline. Cells already induced by noradrenaline assumed their control appearance promptly after addition of colchicine. These observations suggest that polymerisation of the microtubules is involved in the formation of processes in C6 cells.

The morphological change in C6 cells could be observed 30 min after addition of noradrenaline (Fig. 2). At this time about 20% of the cells showed the characteristic processes. The fraction of morphologically altered cells then increased approximately linearly with time. At 4 h more than 90% of all cells

assumed the new morphology. From this time on, more and more cells looked normal once again; and 8 h after the application of noradrenaline all cells were morphologically indistinguishable from untreated cells.

In separate experiments I measured the cyclic AMP level in the cells during their morphological modulation (Fig. 2). Untreated cells had a basal level of 10 ± 4 pmol per mg protein, which is similar to that found in many tissues, including brain. But after treatment with noradrenaline the cyclic AMP concentrations increased strikingly, more than 100-fold, in agreement with previous observations by Gilman and Nirenberg¹. After 30 min the cyclic nucleotide concentration began to decrease and at 4 h it reached the 2–3-fold basal levels. The cyclic AMP level in the cells dropped to the basal value at 8 h.

The data of Fig. 2 show that most of the cells are morphologically altered when the cyclic AMP level has already dropped to almost basal levels (at 4 h). This time course indicates that both effects of noradrenaline are transient. Moreover, I have

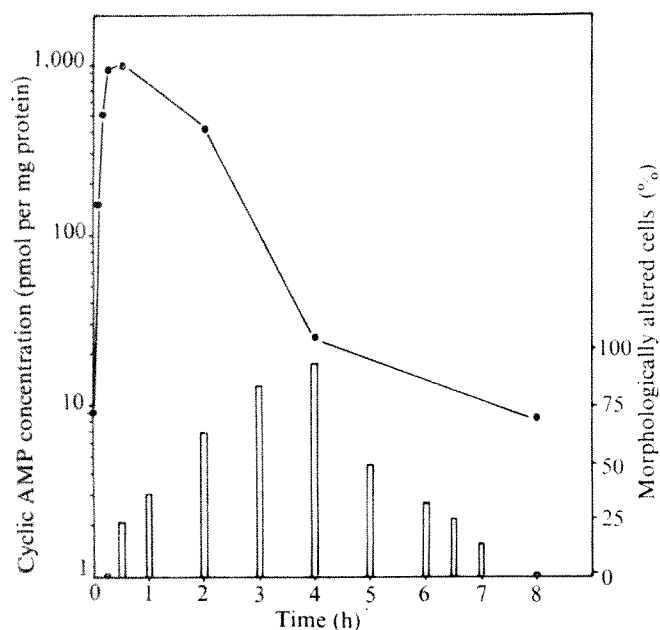


Fig. 2 Time course of morphological alteration (bars) and cyclic AMP concentration (black circles) in nucleated C6 cells induced by 10^{-6} M noradrenaline. Parallel cultures of C6 cells grown in 6 cm dishes (Greiner) were inspected for morphological alteration. The intracellular cyclic AMP concentration was then determined in the parallel culture. Before the experiment was started, C6 cells were incubated in medium for 48 h after replating. The cells were fixed with 100% methanol and stained with Giemsa. A total of 500 cells per dish were examined for the type of morphological alterations shown in Fig. 1. For determination of cyclic AMP the medium was quickly removed by aspiration and the cells were washed twice with PBS at 37 °C and fixed with 2 ml of ice-cold 5% trichloroacetic acid. The cells were then scraped off the dish. The protein debris was removed by centrifugation and the protein concentration was determined by the method of Lowry, using bovine serum albumin (Boehringer Mannheim) as standard. The supernatant was extracted five times with ether, which was presaturated with HCl-acidified water. The samples were then freeze-dried. The dry residue was suspended in 0.2 ml of 0.2 M sodium acetate buffer, pH 4. The cyclic AMP concentration was determined according to Gilman⁵.

found that the cells did not respond to a second treatment with noradrenaline for at least 60 h. After this refractory period, noradrenaline once again induced the characteristic modulation of cyclic AMP and morphology. Addition of dibutyryl cyclic AMP (Boehringer Mannheim, 1 mM) plus theophylline (Sigma, 0.1 mM) also induced the morphological change in C6 cells.

Taken together, these results suggest that noradrenaline stimulates by way of the β -adrenergic receptor, the adenylate cyclase of C6 cells, which then acquire a morphology similar to that of the astrocytes in brain tissues. Both changes are transient and are followed by a long refractory period. There are some reports^{6,7,8} in which cyclic AMP has been implicated in tubulin polymerisation in cells, but not in a cell-free system⁹. The morphological alteration in C6 cells is prevented by colchicine, again indicating the participation of microtubules. This was directly shown for C6 cells after stimulation by noradrenaline, (Weber, personal communication) using the immunofluorescence technique for specific visualisation of cytoplasmic microtubules¹⁰.

My experiments and other reports^{7,11,12} demonstrate that the information necessary for normal cell shape and morphological change is preserved in the cytoplasm of cells enucleated by cytochalasin B. The system described here may be useful for seeking a possible causal relationship between cyclic AMP and polymerisation of microtubules.

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Genetically determined defect of Schwann cell basement membrane in dystrophic mouse

FOR more than a century there has been controversy as to whether Schwann cells are able to synthesise collagen and the collagen-related basement membrane^{1,2}. Recent evidence provides more direct proof that Schwann cells are indeed equipped to synthesise collagen and probably also a basement membrane in the same way as other cells of epithelial and neuroectodermal origin^{3–5}. Observations in various pathological situations, including regeneration after crush injury^{6,7} and in peripheral nerve ischaemia⁸ indicate that axon regrowth and Schwann cell migration occur along scaffolding consisting of basement membranes and endoneurial collagen fibres. The role of the basement membrane and the endoneurial collagen in normal myelination has, however, remained largely unknown. It has been generally accepted that these extracellular elements provide not only mechanical support

to peripheral nerves, but also that they are partly responsible for the relative lack of developmental faults in peripheral myelination⁹. A recent report¹⁰ suggests that the acquisition of a basement membrane by a Schwann cell occurs at the time that the cell establishes a permanent relationship with an axon. It is not known, however, whether the association of the axon with the Schwann cell induces the latter to synthesise basement membrane, or whether the Schwann cell only establishes a relationship with an axon once the Schwann cell has acquired a basement membrane.

There are at least three ways of investigating this problem: (1) by making serial observations during embryogenesis; (2) by studying the effects of selective damage to basement membrane and collagenous elements on myelination; or (3) by studying the mutant dystrophic mouse, in which areas of apparent early arrest of Schwann cell development and myelinogenesis are known to occur, particularly in the proximal peripheral nervous system^{11–13}. Our observations on the dystrophic mouse indicate that a genetically determined partial deficiency of basement membrane is present in the Schwann cells of the peripheral nervous system, and suggest that the Schwann cells develop basement membrane before establishing a definitive relationship with the axons.

Dystrophic (*dy/dy*) mice of 1, 2, 3 and 5 months of age were studied. Phenotypically normal littermates of the dystrophic animals (+/?) and C57BL mice were used as controls for each age group. They were prepared for electron microscopy as reported previously¹¹. Segments of the sciatic nerve 4–5 mm above the sciatic notch were studied at all time points, and at 3 months of age the common peroneal nerve was also examined.

A consistent finding in all dystrophic mice was a patchy deficiency of the basement membrane of Schwann cells of fibres which were myelinated (Fig. 1). The basement membrane was completely absent in amyelinated zones. In longitudinal sections, myelinated fibres entering an area of amyelination lost not only their Schwann cell covering and myelin sheath, but also the associated patchy basement membrane (Fig. 2). Some of the myelinated fibres had abnormally long bare nodal segments, without the usual basement membrane and Schwann cell pseudopodial covering. The abnormally long bare nodes are probably responsible for the 25% reduction in the sciatic nerve conduction velocity found by Huizar *et al.*¹⁴. Skin basal cells, skeletal muscle fibres, and endoneurial blood vessels showed continuous basement membrane of normal thickness. The basement membrane of the normal littermates (+/?) was normal, and identical to that in the normal C57BL mice. The defect of basement membrane was thus inherited in an autosomal recessive manner in these dystrophic mice, and presumably the gene responsible for the dystrophy is also responsible for the basement membrane defect.

Peripheral nerve development in the normal foetus involves migration of Schwann cells around bundles of axons, the progressive division of these bundles into smaller and smaller groups by proliferating Schwann cells, and the eventual segregation and enwrapping of axons by Schwann cells^{15–17}. The exact final relationship depends on whether the axon is to become a myelinated or an unmyelinated fibre. The observations of Webster and colleagues¹⁰ on tadpole nerve indicate that migrating Schwann cells lack basement membrane, which only forms when the Schwann cell establishes a permanent relationship with an axon. In mammalian foetal nerve the Schwann cells taking part in the segregation of axon bundles already have basement membranes^{18,19}. Our studies of the mutant dystrophic mouse show that Schwann cell basement membrane is patchily deficient throughout the peripheral nervous system, and that uncommitted (presumably Schwann) cells around amyelinated nerve fibre bundles in the nerve roots have a similar, and sometimes more marked, deficiency of basement membranes. This basement membrane deficiency is probably the result of impaired Schwann cell function, rather than of an inadequate axonal signal to the Schwann cell. The basement membrane deficiency in the

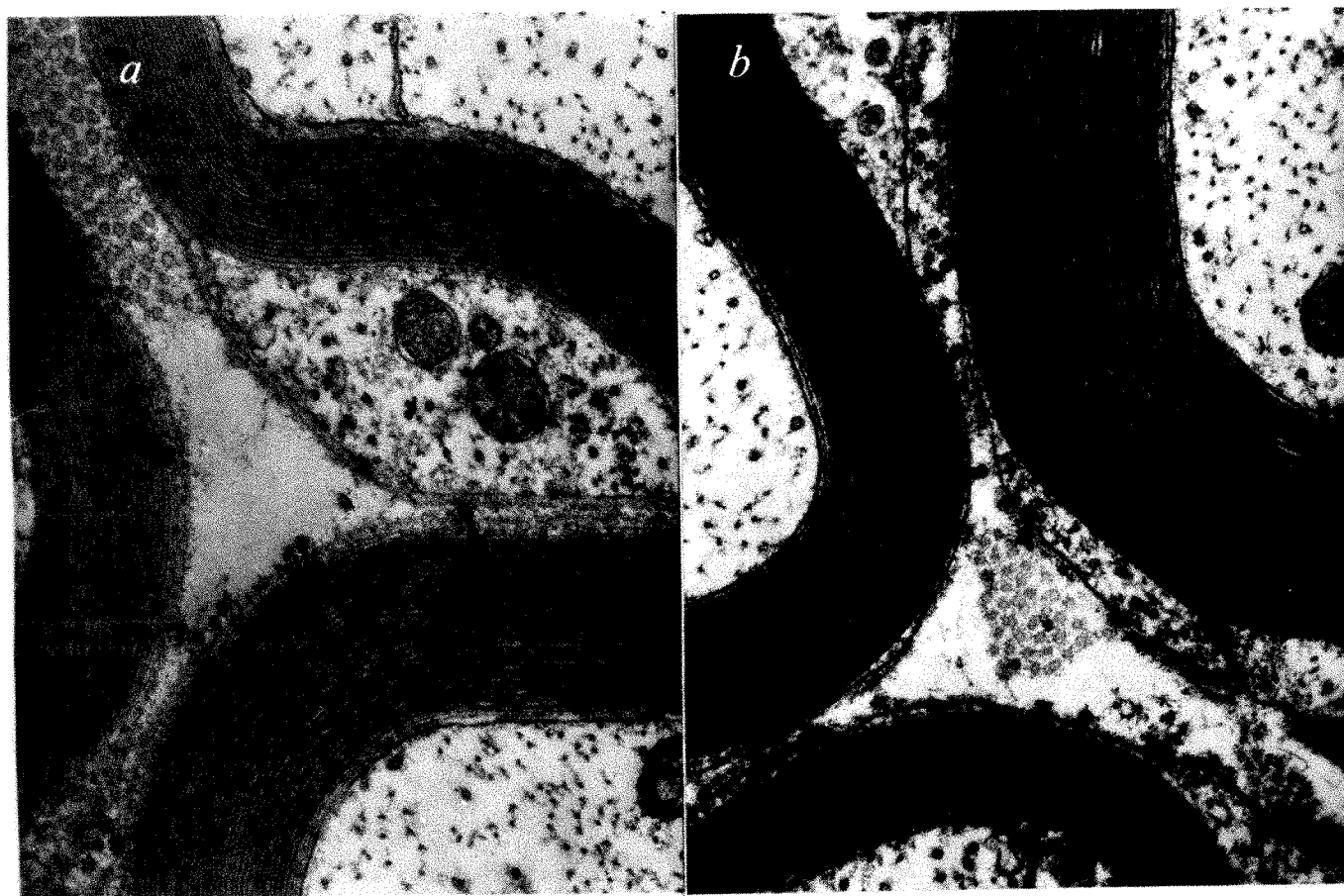


Fig. 1 Two-month-old mouse upper sciatic nerve showing portions of 3 myelinated nerve fibres. *a*, Normal C57BL; *b*, *dy/dy* mouse. In *a* the basement membrane over the Schwann cells is complete, and the minimum separation of 2 Schwann cell plasma membranes is 78 nm. In *b* the dystrophic basement membrane is patchy and incomplete, which allows the plasma membranes of two Schwann cells to approach to within 55 nm in this figure (middle). In some instances, dystrophic Schwann cells plasma membrane approached close enough to form an intraperiod line. In *b* there is apparently abnormally wide separation of the leaflets of the Schwann cell plasma membrane. $\times 45,000$.

dystrophic mouse may therefore simply represent a deficiency of Schwann cell function; there is already suggestive evidence of other Schwann cell abnormalities including an oligodendroglial-like arrangement of some Schwann cells (E. J., R. M.

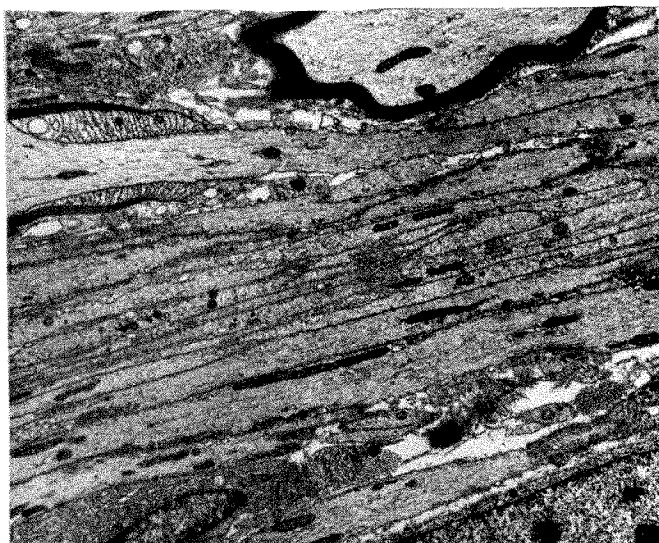
and W. G. B., unpublished), and of aberrant axonal enwrapment and myelination¹¹.

It is possible to advance an hypothesis relating the peripheral nervous system abnormalities to the basement membrane deficiency. Let us suppose that synthesis of the basement membrane were necessary to terminate Schwann cell migration, and to establish a permanent relationship between the Schwann cell and an axon. The decreased ability to synthesise basement membrane characteristics of the dystrophic mutant Schwann cell would result in a greater tendency for Schwann cells to migrate further from the neural crest. Fewer Schwann cells would therefore come to rest in the spinal roots, and those which did settle, both in the roots and more peripheral parts of the nervous system, would show a decreased capacity for axonal enwrapment and myelinogenesis.

There are, however, still some features of the dystrophic mouse peripheral nervous system which are not fully explained by this hypothesis. These include the exact distribution of the neural abnormality, the presence of uncommitted Schwann cells around myelinated axons, and the aberrant myelination in the spinal roots^{11,12}.

To the best of our knowledge this is the first reported incidence of a genetically determined deficiency of basement membrane production of Schwann cells, though abnormalities of the basement membrane have been incriminated in other diseases. In Alport's syndrome (hereditary nephropathy with nerve deafness), the basement membrane of renal glomeruli shows focal thickening, thinning and splitting, and this abnormality is genetically determined²⁰. Deficiency of epithelial basement membrane is believed to be of importance in the invasiveness of carcinoma^{21,23}, and has also been reported in dermatitis herpetiformis²⁴. The Schwann cell of the mutant dystrophic mouse may therefore prove to be an equally useful

Fig. 2 Two-month-old *dy/dy* mouse upper sciatic nerve showing an axon in longitudinal section. To the left is a paranodal termination of the Schwann cell cytoplasm and its myelin sheath. In the middle and right, the axon is naked with neither Schwann cell nor basement membrane covering. $\times 4,500$.



tool to those interested in basement membranes, peripheral nerve development and oncology.

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Mosaicism in organisation of concanavalin A receptors on surface membrane of mouse egg

PLANT lectins have proved to be useful probes of the surface properties of a variety of cells and membranes, providing data on the relative mobility of intramembranous macromolecules in different conditions¹ and on the organisation of oligosaccharides on the cell surface. We report here the use of fluorescent concanavalin A (con A) as a probe to analyse the surface properties of the unfertilised and fertilised mouse egg. Similar experiments were carried out independently at both Cambridge and Birmingham and the results are combined.

The fluorescent staining pattern (for details, see Fig. 1) of unfertilised eggs was not uniform. An area of weak or absent staining, designated the 'negative area', was consistently detected, occupied approximately one-fifth of the egg's surface and was associated in some but not all eggs with a slight distension of the cytoplasmic membrane (Figs 1a and 2). Cytological investigation and vital staining with acridine orange revealed that the negative area was consistently associated with the underlying second metaphase spindle. After incubation at 20 or 37 °C the negative area was separated from positively stained membrane by a sharply defined boundary line (Fig. 1b), but the boundary appeared more diffuse after incubation at 4 °C. The higher temperatures also affected the pattern of staining within the positive area converting it from a dullish even fluorescent staining to a bright patchy staining pattern at concentrations of con A in excess of 50 µg ml⁻¹ (Fig. 1c).

Various drugs were examined for their effects on the staining pattern. Preincubation in neuraminidase (50 or 100 IU ml⁻¹, Behringwerke) did not affect the staining pattern. Sodium azide at 0.5 × 10⁻² M tended to exaggerate the punctate staining of the positive area and caused the area

of negative stain to contract in size (Fig. 1c) and in many eggs to disappear altogether (Fig. 1d). The progressive loss of negatively staining membrane was often accompanied by a breakdown in sharpness of the interface between the two areas. Essentially identical results were obtained with 1, 10 or 100 µg ml⁻¹ cytochalasin B. Dimethylsulphoxide (DMSO) at equivalent dilution to that used to dissolve the cytochalasin B had no effect. The effect produced by both azide and cytochalasin B was observed at whatever stage in the protocol the drug was added, but was more marked if the drug was added before and during incubation with con A than if added after incubation. The effect was partially reversible, removal of cytochalasin B leading to an increased incidence of larger negative areas amongst eggs. Colcemid at doses from 0.36-36.00 µg ml⁻¹ had no effect on the staining pattern. Colcemid, cytochalasin B, DMSO and azide all caused rounding up of the eggs to spherical shapes, and cytochalasin B and DMSO, but not azide, caused a slight displacement of the metaphase spindle away from the surface membrane.

Newly fertilised eggs at pronuclear stages and earlier were also examined for reaction with con A. The egg was positive over the whole membrane, displaying a patchy staining pattern when incubated at 20 or 37 °C. The patchy, as distinct from smooth, staining pattern on fertilised eggs was induced by concentrations of con A one-fourth the minimum dilution effective for unfertilised eggs. An association was noted between a patchy staining pattern and the agglutination of eggs by con A, as both occurred more with fertilised than unfertilised eggs, with azide or cytochalasin B present and at 37 but not at 4 °C. The membrane overlying the midbody connecting the egg to the polar body was intensely positive, but the polar body was always either negative or had a small cup-shaped region of positive stain at its junction with the midbody (Figs 1e and 2).

These experiments demonstrate that the plasma membrane of the unfertilised mouse egg seems to be a mosaic of two distinct regions, one staining with fluorescent con A and constituting most of the egg's surface, the other on which little or no stain is detectable, overlying the second metaphase spindle and specifically isolated as the membrane of the second polar body. This pattern is susceptible to disorganisation by azide, which inhibits energy-dependent processes, and by cytochalasin B, which affects surface membrane activity and microfilament organisation. As it seems probable that energy-dependent microfilament activity is in part responsible for organising the contractile ring of the cleavage furrow which partitions the cell at division³, the mosaicism in the organisation of the surface con A-binding groups may reflect an underlying intracellular organisation dependent on microfilaments.

The nature of the membrane mosaicism is not clear from these experiments but could exist at either a structural or molecular level. A mosaicism of the underlying intracellular organelles is well established from ultrastructural studies, which have shown the cytoplasm in the spindle region to be relatively devoid of cortical granules, multivesicular bodies and membrane laminae². The mosaicism of con A binding seen at the light microscope level may be a molecular correlate of this polarity. The staining seems to be a genuine surface phenomenon and not the result of a selective pinocytosis in the positive area, as preincubation in azide and cytochalasin B caused extension of staining over the whole area rather than loss or reduction of positive staining. Organisation of the membrane of the positive area into microvilli may increase the fluorescence intensity either by increasing the effective density of con A-binding sites through corrugation of the surface or by effecting a reorganisation of binding sites within the membrane itself⁴. The intense staining of the midbody region is most probably caused by extensive surface corrugation²,

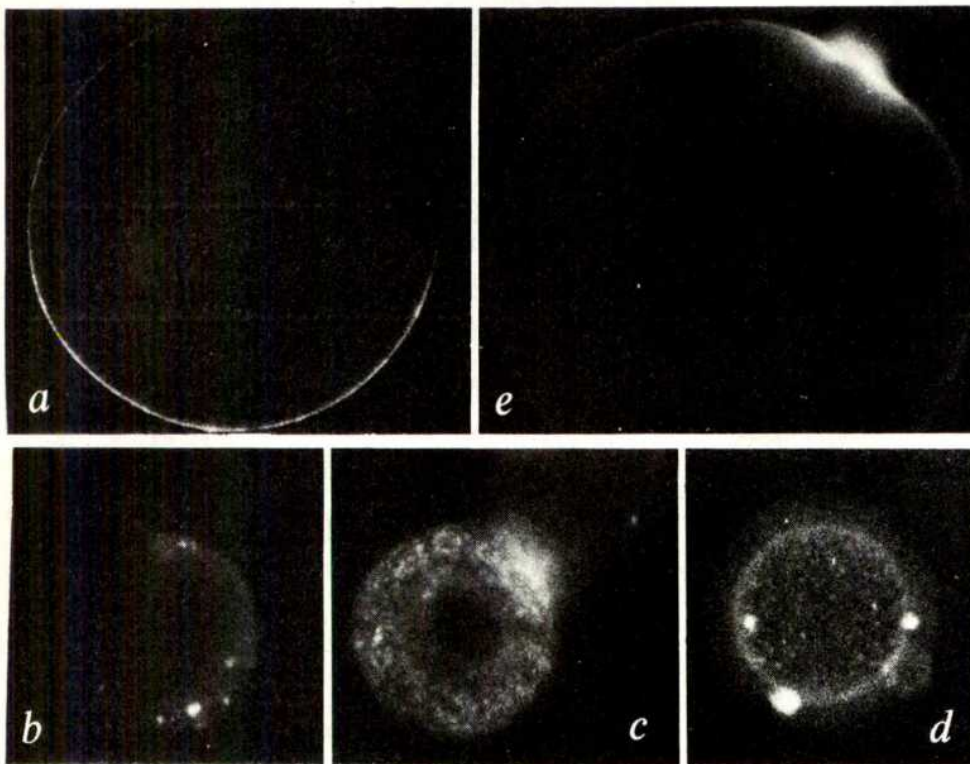


Fig. 1 Fertilised and unfertilised eggs were recovered from outbred CFLP and LACA mice within 2–3 h of superovulation. The cumulus cells were removed with hyaluronidase (Koch-Light 50 IU ml⁻¹ in phosphate-buffered saline with 10% polyvinylpyrrolidone), and the zona pellucida was removed with Pronase (0.5%, Tris-citrate buffer pH 7.0). Eggs were washed in medium PB1 (ref. 5) and used immediately. Eggs (5–10) were placed under oil in each well of a microtitre dish (Baird and Tatlock, tissue-typing slide catalogue 77 403/0522). Reagents were added by washing the eggs in each well with three changes of PB1 + reagent. Incubations were carried out at 4, 20 or 37 °C for 20–30 min. After incubation the eggs were washed in several changes of medium. A coverslip was applied and the eggs viewed on a Zeiss Photomicroscope (light source HBO200; excitation filters KP490 and 500, and LP455; barrier filters KP560 and LP520). Photographs were taken using Kodak recording film 2475. Fluorescein-labelled con A was obtained from Miles Laboratories and also synthesised in the laboratory. The activity was titrated and a final concentration of 50 µg ml⁻¹ was used in most experiments. α -Methyl-D-mannoside blocked staining. *a*, Unfertilised mouse egg incubated with fluorescein-labelled con A (25 µg ml⁻¹) at 20 °C for 30 min. Note absence of staining at one pole of egg ($\times 264$). *b*, As *a* but at lower magnification to show the clearly defined line of demarcation separating positive and negative areas ($\times 80$). *c*, Unfertilised egg stained with fluorescein-labelled con A in the presence of azide. Note how the size of the negative patch has contracted ($\times 80$). *d*, As *c* but showing an egg in which no negative area is visible ($\times 80$). *e*, Fertilised egg showing positive stain over whole surface of egg, intense stain of midbody but absence of stain in adjacent polar body ($\times 264$).

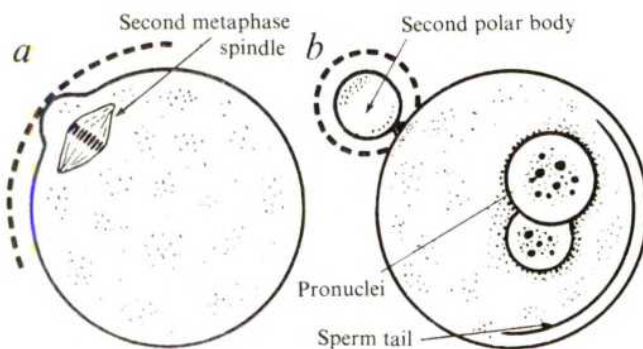


Fig. 2 Diagram indicating organisation of unfertilised (*a*) and fertilised eggs (*b*). Dotted outline indicates region of negative area.

and in preliminary ultrastructural studies we have found a reduced incidence of microvilli over the metaphase spindle. These aspects are being explored further at the ultrastructural level using ferritin-labelled con A.

Mosaicism at a molecular level could arise for several reasons. Masking of con A-binding residues in the negative area by neuraminidase-sensitive material has been excluded experimentally. Mannose-bearing oligosaccharides could be selectively excluded from the negative area. Alternatively, the oligosaccharides could be present but immobilised perhaps by way of microfilaments in such a way as to prevent cross linking by con A and their visualisation in patches¹, as evidently occurs elsewhere on the membrane. Note that in these studies the zonae pellucidae were removed by Pronase, which could have effects on the susceptibility to patching of the egg membrane.

Whatever the basis of the mosaicism, con A promises to be a useful probe of surface properties of the egg in different conditions. Such observations may also be relevant to the mechanisms of sperm-egg interaction. We have observed that sperm attachment *in vitro* to the negatively staining areas of zona-free unfertilised eggs is rare (M.H.J., and D.E., unpublished). The mosaicism of con A receptors may reflect an underlying membrane organisation, pre-

venting sperm attachment and fusion at a site at which extrusion of the second polar body may be impaired or abnormal extrusion of the fertilising sperm head may occur. Haploid or triploid states might thereby be avoided.

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Release of immunoreactive luteinising hormone-releasing hormone and thyrotrophin-releasing hormone from hypothalamic synaptosomes

THE hypophysiotropic hypothalamus regulates the secretion of the anterior pituitary gland through the production and release of specific peptide hormones. Luteinising hormone-releasing hormone (LHRH) and thyrotrophin-releasing hormone (TRH) are two such peptides which have been fully characterised and synthesised^{1,2}. Studies on intact animals have revealed the importance of various neurotransmitters and hormones in the modulation of hypothalamic LHRH and TRH secretion but little is known of the cellular mechanisms involved in such control, especially at the neurosecretory nerve terminal. We have described^{3,4} the use of nerve endings (synaptosomes) isolated from the mammalian hypothalamus to investigate factors which influence the release of corticotrophin-releasing factor (CRF). As this factor is uncharacterised, a major limitation of this work has been the use of bioassay techniques to measure indirectly corticotrophin-releasing activity. In the study described here radioimmunoassays have been used to measure the release of immunoreactive LHRH and TRH from synaptosomes prepared from rat and sheep hypothalami, and we report the influence of neurotransmitters and steroid hormones on these processes.

Nerve endings were isolated from hypothalami (600–800 mg, wet weight, sheep; 30–50 mg, rat) by the method described previously⁴ and suspended in Krebs bicarbonate medium containing 10 mM glucose at a concentration of 1 and 3 hypothalamic equivalents per ml for sheep and rat, respectively. In some experiments sheep hypothalami were dissected into three regions: the median eminence (50–100 mg); a more dorsal, medial periventricular region (250–300 mg) and the remainder of the hypothalamus. These fragments were collected and treated separately. Samples (1 ml) of synaptosome suspensions were incubated for 30 min at 37 °C in capped glass vials. Electrical field stimulation was carried out during the last 20 min of incubation by passing alternating square wave pulses through concentric gold ring electrodes immersed in the synaptosome suspensions. The parameters of this electrical stimulation have been described previously⁴. Potassium stimulation was achieved by increasing the potassium concentration of the incubation medium to 60 mM during the last 10 min of incubation. In further experiments neurotransmitters or steroid hormones were added directly to the vials in 20 µl of incubation medium. After incubation the synaptosomes were removed by centrifugation, and the supernatants were rapidly frozen immediately and stored at –20 °C for 1–5 d before measurement of the LHRH and TRH content by radioimmunoassays, which have been described in detail elsewhere^{5,6}.

Synaptosomes isolated from whole sheep hypothalami released both LHRH and TRH to the medium (Table 1). Unstimulated preparations released almost fifteen times more TRH than LHRH, estimated on a molar basis, whereas electrical stimulation produced a sevenfold increase in LHRH and a fivefold increase in TRH in the medium. In contrast, depolarising concentrations of potassium had no significant effect on the release of either hormone. This difference in response to the two stimuli is surprising: it is possible either that the transitory depolarisations produced by electrical pulses are a more effective stimulus than the tonic depolarisation produced by potassium, or alternatively that elevated potassium levels may in some way stimulate the reuptake or degradation of the

Table 1 Release of immunoreactive LHRH and TRH by synaptosomes isolated from sheep hypothalami

Test	LHRH	TRH
Control	271 ± 24 (11)	1,536 ± 182 (14)
60 mM K ⁺	289 ± 23 (12)	1,617 ± 148 (9)
Electrical field stimulation	2,018 ± 696 (12)*	7,217 ± 1,060 (12)†

Values represent pg of immunoreactive hormone (mean ± s.e.m.; number of experiments shown in parentheses) released by 1 ml of a suspension of hypothalamic synaptosomes (equivalent to one hypothalamus) during 30 min incubation in Krebs bicarbonate medium at 37 °C. Electrical field stimulation and potassium stimulation were for the last 20 min and 10 min respectively. Student's *t* test: **P* < 0.02, †*P* < 0.001.

hormones released to the medium. Synaptosomes prepared from whole hypothalami of sheep were incubated in the presence of a range of concentrations of neurotransmitters known to be present in the mammalian hypothalamus, particularly in the median eminence (Table 2). Dopamine at concentrations of 10⁻¹⁰ M or more stimulated the release of LHRH but was without effect on TRH release. In contrast, 5-hydroxytryptamine (5-HT) at 10⁻⁸ M had no effect on LHRH release but significantly inhibited the release of TRH. Acetylcholine, noradrenaline and adrenaline were without effect on either LHRH or TRH release.

Nerve endings prepared from the three dissected regions of the sheep hypothalamus were also incubated and electrically stimulated or tested with dopamine (10⁻⁸ M) and the results are shown in Table 3. The levels of LHRH and TRH released from control and electrically stimulated preparations from the median eminence resembled those obtained with preparations derived from whole hypothalamus. The incubation of median eminence synaptosomes in the presence of dopamine stimulated the release of both LHRH and TRH. The absence of an effect of dopamine on TRH release in preparations from the whole hypothalamus may thus reflect the heterogeneous nature of the synaptosome population and the possibility that inhibitory neurotransmitters or other factors can block the stimulation induced by dopamine.

Synaptosomes prepared from the central periventricular region and the remainder of the hypothalamus released low

Table 2 Effect of neurotransmitters on the release of immunoreactive LHRH and TRH by synaptosomes isolated from sheep hypothalami

Neurotransmitter	Concentration (M)	LHRH	TRH
Control		271 ± 24 (11)	1,536 ± 182 (14)
Dopamine	10 ⁻¹²	253 ± 50 (5)	1,752 ± 180 (5)
	10 ⁻¹⁰	555 ± 130 (10)*	1,361 ± 130 (9)
	10 ⁻⁸	537 ± 180 (6)	1,356 ± 184 (6)
	10 ⁻⁶	560 ± 113 (3)*	1,181 ± 335 (3)
Noradrenaline	10 ⁻¹²	210 ± 16 (7)	1,967 ± 1,250 (3)
	10 ⁻¹⁰	271 ± 46 (9)	1,320 ± 259 (5)
	10 ⁻⁸	308 ± 22 (6)	1,253 ± 64 (4)
	10 ⁻⁶	243 ± 41 (3)	1,327 ± 156 (4)
5-HT	10 ⁻¹²	201 ± 7 (3)	1,968 ± 143 (3)
	10 ⁻¹⁰	234 ± 26 (6)	1,918 ± 172 (6)
	10 ⁻⁸	273 ± 52 (3)	666 ± 82†
	10 ⁻⁶	207 ± 23 (3)	660 ± 77†
Acetylcholine	10 ⁻⁸	253 ± 40 (6)	1,600 ± 206 (3)
Adrenaline	10 ⁻⁸	289 ± 37 (4)	1,708 ± 196 (4)

Values represent pg of immunoreactive hormone (mean ± s.e.m., number of experiments shown in parentheses) released by 1 ml of a suspension of hypothalamic synaptosomes (equivalent to one hypothalamus) during 30 min incubation in Krebs bicarbonate medium at 37 °C. Neurotransmitters were added in 20 µl of medium at the start of each incubation. Student's *t* test: **P* < 0.05, †*P* < 0.001.

Table 3 Release of immunoreactive LHRH and TRH by synaptosomes isolated from different regions of the sheep hypothalamus

Test	Median eminence	LHRH		Median eminence	TRH	
		Periventricular hypothalamus	Remainder of hypothalamus		Periventricular hypothalamus	Remainder of hypothalamus
Control	169 ± 20 (9)	64 ± 30 (6)	47 ± 10 (6)	1,222 ± 223 (9)	853 ± 307 (6)	720 ± 126 (6)
Electrical stimulation	601 ± 105 (9)*	153 ± 40 (7)§	95 ± 20 (8)§	5,044 ± 909 (9)*	3,080 ± 494 (7)†	3,260 ± 680† (5)
Dopamine (10 ⁻⁸ M)	565 ± 154 (11)‡	46 ± 6 (8)	39 ± 9 (8)	4,854 ± 927 (11)†	1,213 ± 200 (8)	1,115 ± 130 (7)

Values represent pg of immunoreactive hormone (mean ± s.e.m., number of experiments in parentheses) released to 1 ml of a suspension of hypothalamic synaptosomes (equivalent to one hypothalamus) prepared from three different regions as described in the text and incubated for 30 min in Krebs bicarbonate medium at 37 °C. Electrical stimulation was carried out during the last 20 min of incubation while dopamine was added at the start of the incubation period: Student's *t* test: **P* < 0.001, †*P* < 0.005, ‡*P* < 0.02, §*P* < 0.05—tested against control results for each preparation.

levels of LHRH whereas the amounts of TRH were higher and comparable with those released from median eminence preparations. Electrical stimulation caused release of both hormones and the responses were of similar magnitude to those obtained with synaptosomes prepared from the median eminence. In contrast with the latter preparation, dopamine added to synaptosomes obtained from the periventricular region or remainder of the hypothalamus did not result in the increased release of LHRH or TRH. This may indicate that the LHRH and TRH-containing nerve endings which terminate outside the median eminence do not possess receptors for dopamine, or that these preparations contain an inhibitory factor(s) which may block the response to this transmitter.

The inhibitory effect of 5-HT on TRH release and the stimulatory effect of dopamine on LHRH and TRH release from synaptosomes prepared from the median eminence are consistent with findings obtained in the whole animal^{7,8}, and with the isolated hypophysiotropic hypothalamus incubated *in vitro*⁹⁻¹¹. These results, together with our finding³ that acetylcholine stimulates and dopamine inhibits the release of CRF from hypothalamic nerve endings, suggest that the neurotransmitters present in the median eminence have a specific role in modulating the release of hypophysiotropic hormones. The extent and physiological significance of such control mechanisms at the neurosecretory nerve terminal remain to be established but further evidence for integration at this level has been obtained from experiments in which the effects of gonadal steroids on the release of LHRH from synaptosomes prepared from the rat hypothalamus have been investigated.

Adult female Sprague-Dawley rats (250 g body wt) were treated with 17- β -oestradiol and progesterone, alone and in combination, administered as a microparticulate suspension in the drinking water (10 μ g ml⁻¹) for 20 h (average ingested dose 150 μ g per rat) before killing, removal of the hypothalamus and preparation of synaptosomes. The same steroids were tested *in vitro* at a concentration of 10⁻⁸ M for a direct

action on hypothalamic synaptosomes prepared from untreated rats. In all cases tested the release of TRH was unaffected by these procedures. Table 4 shows that synaptosomes prepared from the rat hypothalamus released much smaller amounts of LHRH than the sheep preparations (14 pg per hypothalamic equivalent compared with 271 pg) although these amounts are in proportion to the weights of original tissue. Pretreatment with oestradiol or progesterone *in vivo* resulted in a two- to threefold increased release of LHRH, whereas simultaneous pretreatment with both hormones produced a marked inhibition. When added directly *in vitro*, oestradiol (10⁻⁸ M) alone had no apparent effect on the nerve endings, although progesterone (10⁻⁸ M) alone and in the presence of oestradiol resulted in an increased release of LHRH. The rats used in these experiments were mature females in all stages of the oestrous cycle and the results can only confirm the complexity of interactions between gonadal steroids and the synthesis and release of LHRH. The *in vitro* results, however, suggest that at least part of the feedback effects of gonadal steroids may occur at the neurosecretory nerve ending; an effect analogous to our previous finding³ that glucocorticoids inhibit the release of CRF from hypothalamic synaptosomes. To clarify further the effects of gonadal steroids *in vitro* shown in Table 4, the effects of 17- β -oestradiol and progesterone on the basal release of LHRH from hypothalamic synaptosomes prepared from female rats taken from specific stages of the oestrous cycle are now being examined. Like the actions of neurotransmitters, the effects of gonadal steroids administered *in vitro* seem to be specific. Thus progesterone increases the release of LHRH, but has no effect on TRH release and at high levels inhibits the release of CRF induced by electrical stimulation. These findings support our view³ that steroids may influence neural processes through mechanisms which do not involve the classical nuclear receptor for such hormones. Studies are in progress to establish whether these effects could be mediated through the action of cyclic nucleotides and mechanisms involving protein synthesis.

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Table 4 Effect of gonadal steroids on release of immunoreactive LHRH by synaptosomes isolated from the female rat hypothalamus

Test	LHRH	
	<i>In vivo</i> pretreatment	Added <i>in vitro</i>
Oestradiol	121 ± 35 (6)*	34 ± 18 (5)
Progesterone	92 ± 16 (5)†	135 ± 48 (6)*
Oestradiol + progesterone	17 ± 4 (5)‡	118 ± 42 (3)
Untreated controls	43 ± 7 (8)	

Values represent pg of immunoreactive LHRH (mean ± s.e.m., number of experiments in parentheses) released by 1-ml suspension of hypothalamic synaptosomes (equivalent to three hypothalami) during 30 min incubation in Krebs bicarbonate medium at 37 °C. For pretreatment (*in vivo*) rats were administered steroid (10 μ g ml⁻¹) in drinking water for 20 h before isolation of synaptosomes. Steroids (10⁻⁸ M) were added directly to synaptosome suspensions for experiments (*in vitro*). Student's *t* test, comparison with untreated control values: **P* < 0.05, †*P* < 0.02, ‡*P* < 0.005.

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Isolation and structure of urogastrone and its relationship to epidermal growth factor

THE clinical observation of the very low incidence of peptic ulceration during pregnancy led to the finding that extracts of the urine of pregnant women had a beneficial effect on experimental ulcers in dogs¹. It was later reported that not only pregnancy urine but also that from all females and males contained a potent inhibitor of gastric acid secretion^{2,3}. Sandweiss⁴ named the anti-ulcer factor anthelone and regarded it as having a therapeutic effect on ulcers without depressing gastric acid secretion. The antisecretory agent was thought to be a separate entity and was called urogastrone because its actions resembled those of the postulated duodenal hormone enterogastrone⁵. Subsequent work amply demonstrated urogastrone action, but the existence of anthelone as a separate entity remained less well established. To establish the true nature of these agents, however, and in particular their possible role in the therapeutic control of peptic ulceration it was necessary to make a full chemical identification. Many attempts have been made to isolate urogastrone, and probably the most highly purified sample on record was that obtained by Gregory⁶. This was described as a combination of a golden yellow fluorescent pigment and a protein of relatively low molecular weight. Others have provided additional evidence of a protein structure⁷ but some recent work has led to the suggestion that urogastrone is a high-molecular weight glycoprotein^{8,9}. Nevertheless its exact nature, source¹⁰ and physiological role have remained unknown. Gastric inhibitory effects have also been shown by extracts of animal urine but studies were undertaken on the nature of urogastrone from more readily available human urine.

Highly purified samples of urogastrone from normal male urine have now been obtained by means of a twelve-stage purification process involving ion exchange, partition and gel chromatography¹¹. There seems to be more than one inhibitor of gastric acid secretion in urine but the main active components were eventually obtained in yields of less than 1 mg per 1,000 l of urine. Two closely related products were isolated and for these the original name of urogastrone has been used. The two urogastrones were shown to be water-soluble polypeptides of relatively low molecular weight and the difference between them could be shown by various physical techniques including acrylamide gel electrophoresis (Fig. 1). They caused an intense inhibition of gastric acid secretion in cats and dogs (when tested as described¹² for less pure material obtained previously) and seemed to be biologically indistinguishable. Because of the small amounts of material available, the two peptides were sometimes used together. Doses as low as 0.25 µg kg⁻¹ produced 60–80% inhibition in Heidenhain pouch dogs stimulated to near maximal levels of acid secretion with pentagastrin or histamine. Work with less pure material had established that at doses producing strong inhibition of acid secretion, urogastrone did not affect other secretions such as pancreatic, biliary or salivary secretion and the effects seemed to be quite specific for the stomach. It did not affect blood pressure, pulse rate or body temperature¹³. Also, it did not seem to be inhibiting acid secretion by causing restriction of mucosal blood flow^{13,14}. Urogastrone has been shown to be active in a number of species and, in man, doses of 0.25 µg kg⁻¹ strongly inhibit gastric secretion induced by different stimuli¹⁵. Acid secretion was also

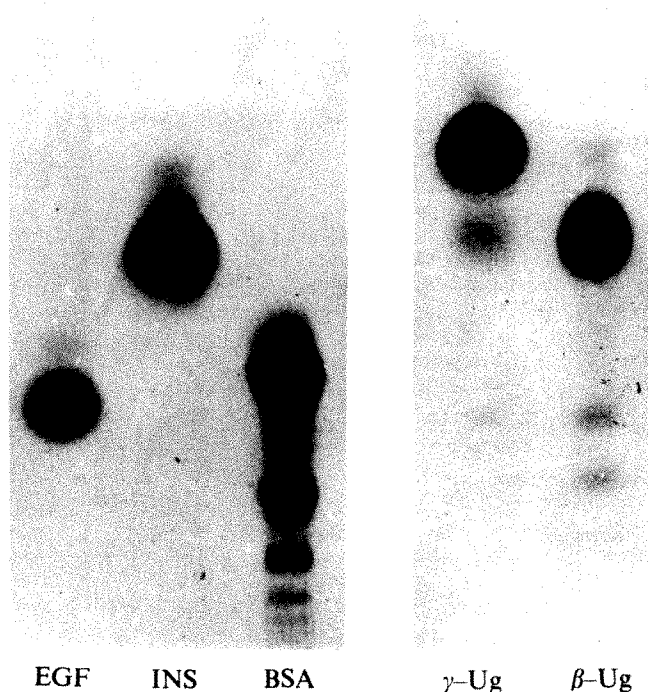
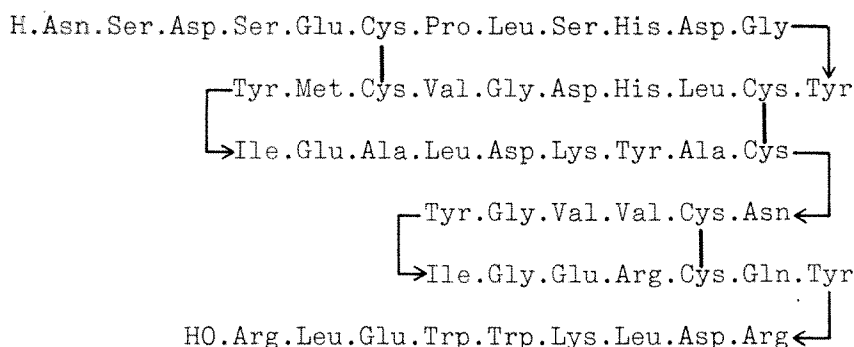


Fig. 1 Thin-layer acrylamide gel electrophoresis at pH 8.9 in which the two urogastrones (β -Ug, γ -Ug) and epidermal growth factor (EGF) are compared with control samples of bovine insulin (INS) and bovine serum albumin (BSA) stained with amido black. The lower edge of the photograph indicates the origin and the upper edge the distance moved by bromophenol blue to the anode.

reduced in patients with Zollinger Ellison syndrome who had massive gastric hypersecretion¹⁶.

The two isolated polypeptides, designated β and γ urogastrone, were found to consist of 53 and 52 amino acid residues respectively. The full structures of these peptides have now been established (Fig. 2) and it was found that the difference resided only in the C-terminal arginine residue which was absent in the γ peptide (H. G., and B. Preston, unpublished). The amino acid sequence was established mainly by degradation of the S-carboxymethylated or S-carboxamidomethylated peptides using trypsin, chymotrypsin, thermolysin or partial acid hydrolysis followed by dansyl Edman degradation of the isolated fragments. The protease derived from *Armillaria mellea* was of particular value, giving specific bond cleavage on the amino side of lysine^{17,18} and providing three fragments from urogastrone derivatives. The positions of the disulphide bonds were established by partial acid degradation of the intact urogastrone molecule.

When this sequence was established it seemed to be quite different from any other known structure and from known gastrointestinal hormones in particular. But a structure has recently been presented for mouse epidermal growth factor (EGF)¹⁹, a polypeptide isolated from the submaxillary glands of male mice which seem to be quite a rich source of biologically active peptides. Early work with this material showed that it possessed the remarkable property of causing premature eye opening when injected daily into newborn mice²⁰ and it has subsequently been shown to stimulate the growth of epithelial cell tissue in a variety of preparations²¹. EGF was isolated as 53 and 51 residue peptides and the published structures showed interesting similarities to urogastrone. Epidermal growth factor was therefore isolated by Cohen's procedure with minor variations and the material obtained had physical properties as described²². The behaviour of EGF on thin-layer acrylamide gel

Fig. 2 The amino acid sequence of β -urogastrone.

electrophoresis was compared with the two urogastrones (Fig. 1); it moves more slowly to the anode at pH 8.9 than the two urogastrones which differ by the single arginine residue. The potency of this preparation was confirmed by its action on newborn mice (Alderley Park Strain). EGF given as subcutaneous injections of 5 μ g in 25 μ l saline d^{-1} caused the eyes to open on day 10 compared with day 13 in the controls²³. It was found subsequently that this material would produce strong inhibition of gastric acid secretion in rats and dogs when given intravenously against exogenous stimuli²³. But the doses of EGF required to induce eye opening ranged from over 300 μ g $kg^{-1} d^{-1}$ initially to about 100 μ g kg^{-1} , whereas inhibition of acid secretion was obtained with less than 0.5 μ g kg^{-1} in dogs and less than 10 μ g kg^{-1} in rats. The time course of inhibition was indistinguishable from that obtained by intravenous injections of urogastrone¹². Maximum inhibition was obtained 15–30 min after injection and secretion then gradually returned to plateau levels after some 90 min. Longer periods of inhibition could be obtained by giving the peptides subcutaneously at rather higher doses. It seemed, therefore, that EGF had biological actions similar to those established for urogastrone and so urogastrone was examined to see whether it would produce the known effects of EGF on newborn mice. At the established dose levels of 5 μ g per mouse d^{-1} it was found that urogastrone would also induce eye opening on day 10 compared with day 13 for the controls (J. M. Bower, unpublished). Although the full spectrum of EGF actions has not been studied there is substantial overlap in the properties of the two polypeptides.

The two peptides show remarkable structural similarities

(Fig. 3). Of the 53 amino acid residues comprising the longer urogastrone and EGF molecules 37 are common to both peptides. The six cysteines occupy the same relative positions and the three disulphide bonds are formed in the same direction. The 16 variable residues occur at intervals along the chains and of these, 14 are interpretable as single base changes in the coding triplets. The two additional pairs are at position 28—Ser, Lys—and position 44—Thr, Tyr. The longest sequences common to both peptides consist of five residues but one of these—the interesting looking C-terminal pentapeptide—does not seem to be necessary for inhibitory activity^{24,25}.

The overall number of changes is perhaps greater than found typically in hormones with similar functions from different species²⁶. There are only four changes in the 51 residues of the insulins of mice and men although guinea pig insulin shows 16 changes when compared with that of man. On the other hand, there are hormone families, the gastrointestinal group of secretin, glucagon, VIP and GIP for example, in which differing biological actions are associated with molecules having common structural features. In this case, secretin differs from glucagon by 14 of the 29 residues²⁷. The structural relationship of urogastrone and epidermal growth factor, however, is supported by apparently identical biological actions.

The salivary glands of species other than the mouse are not a good source of the growth factor²⁰ and only the male mouse provides substantial quantities. Females can be made to produce much larger amounts of EGF, however, as a result of androgen treatment²⁸. This sexual dimorphism does not normally apply to urogastrone and levels in urine are very similar for the human male and non-pregnant female¹⁰. Interestingly, the only

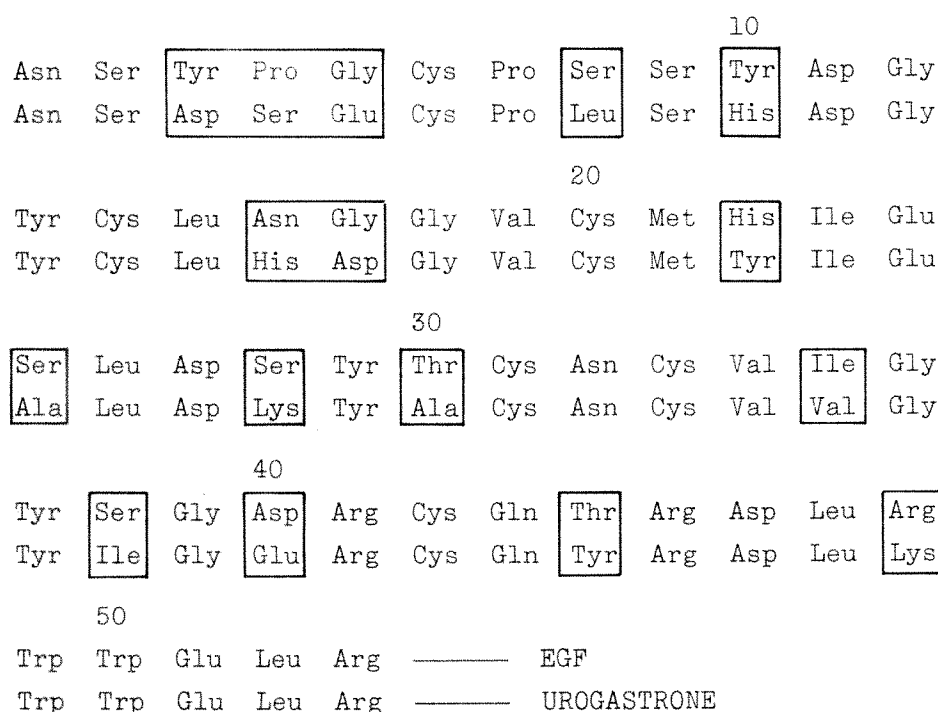


Fig. 3 Comparison of the amino acid sequences of human urogastrone and mouse epidermal growth factor with the changed residues enclosed.

Table 1 Amino acid composition of urogastrone compared with human and mouse epidermal growth factor

Amino acid	β -Urogastrone	Human EGF ³⁰	Mouse EGF ¹⁹
Lys	2	3	0
His	2	2	1
Trp	2	1	2
Arg	3	2	4
Asp	7	7	7
Thr	0	0	2
Ser	3	3	6
Glu	5	5	3
Pro	1	2	2
Gly	4	5	6
Ala	2	2	0
Half-Cys	6	6	6
Val	3	2	2
Met	1	1	1
Ile	2	2	2
Leu	5	4	4
Tyr	5	2	5
Phe	0	0	0
Total	53	49	53

indications of differences in urogastrone excretion lie in the many reports of higher urinary levels during pregnancy, when oestrogen and progesterone levels are raised. A recent study with a mouse EGF immunoassay showed that blood levels of an immunoreactive species could be demonstrated during the early stages of pregnancy in humans but none could be detected in the male or non-pregnant female²⁹. A sensitive radioimmunoassay for human urogastrone as well as EGF has been developed (H. G., J. E. Holmes, and I. R. Willshire, unpublished) and accurate determinations of urogastrone and possible EGF in various human conditions are being made.

Recently, a human epidermal growth factor has been isolated from human pregnancy urine and a total amino acid composition has been given³⁰. These figures are given in Table 1 together with mouse EGF and are compared with the ratios for β -urogastrone. Although there are differences in several of the figures, those for human EGF are not far removed from the urogastrone ratios and may be indicative of two closely related polypeptides. Because the problems of obtaining accurate amino acid ratios with small amounts of material are well known, however, it is probable that urogastrone and human epidermal growth factor are one and the same.

It is worth recalling the definition that Sandweiss applied to anthelone over 30 years ago—that it produced a beneficial effect on experimental ulcers by promoting fibroblastic proliferation and epithelialisation of the mucosa³¹. It seems that the gastric inhibitory polypeptide urogastrone may also have the epithelial growth characteristics once ascribed to anthelone as a separate entity and work now in progress will show whether it may be of value as a therapeutic agent in the treatment of duodenal ulceration.

I thank colleagues who have been involved in different aspects of the work on urogastrone and in particular Mr I. Willshire, Mrs B. Preston, Dr J. Raventos, Mrs E. Haworth, Dr L. Gerring, Miss J. Bower and Mr W. Broadbent.

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Incorporation of GMP into specific tRNA molecules by extracts of Ehrlich ascites tumour cells

ENZYMATIC activities for synthesising four ribohomopolymers, including poly(G), have been found in rat liver, and all are stimulated by RNA^{1,2}. Recently two poly(G) polymerases were isolated from plant cells; one requires poly(G) as a primer³ and the other denatured DNA⁴. We report here the isolation of an enzyme from Ehrlich ascites tumour, which catalyses the incorporation of GMP from GTP into an acid-insoluble product. This enzyme requires specific tRNAs as primers. Our data suggest that the tRNAs which can serve as primers are specific to tumours or tissues infected with tumour viruses.

The GTP incorporating enzyme activity we studied was

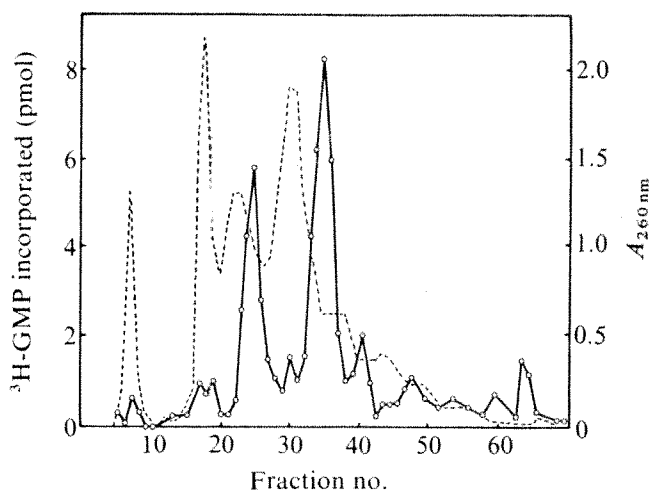


Fig. 1 Primer activity of various tRNAs from Ehrlich ascites tumour. About 3.8 ml of tRNA solution (2.4 mg ml⁻¹) containing 0.25 M NaCl was applied to a 0.6 × 90-cm RPC-5 column, previously equilibrated with buffer (0.01 M Tris-HCl, pH 7.5, 0.01 M Mg(CH₃COO)₂, 0.0003 M 2-mercaptoethanol, 0.4 M NaCl). A 400-ml linear gradient of 0.5–1.0 M NaCl was generated and pumped (about ~20 kg cm⁻²) through the column at about 0.3 ml min⁻¹. 4.7-ml fractions were collected. This experiment was performed at the National Cancer Center Research Institute, through the kindness of Dr S. Nishimura. The absorbance at 260 nm of each fraction was measured (dotted line). Primer activity of each fraction was measured by adding 0.02 ml of each fraction into the standard reaction mixture containing 30 µg of enzyme protein (continuous line).

Table 1 Effects of various nucleic acids offered as primers

		³ H-GMP incorporated				³ H-GMP incorporated	
Primer		(μg)	(pmol)	Primer		(μg)	(pmol)
Experiment 1	Ehrlich ascites tumour			Mouse spleen RNA	10	1.0	
	Whole RNA	10	13.6	liver RNA	10	1.3	
	tRNA	2.5	13.5	Rat spleen RNA	10	<0.5	
	rRNA	10	0.5	liver RNA	10	0.5	
	<i>E. coli</i>			MS2 phage RNA	10	<0.5	
	Whole RNA	10	25.9	GA phage RNA	10	<0.5	
	tRNA	2.5	27.4	Qβ phage RNA	10	<0.5	
	rRNA	10	3.1	SP phage RNA	10	<0.5	
	Methionine tRNA _f	5	< 0.5	FIC phage RNA	10	<0.5	
	Methionine tRNA _m	5	< 0.5	TMV RNA	10	2.0	
	Valine tRNA _i	5	< 0.5	Calf thymus DNA (native)	10	<0.5	
	Phenylalanine tRNA	5	< 0.5	(denatured)	10	<0.5	
	Glutamic acid tRNA _{II}	5	< 0.5			(nmol)	
	Serine tRNA _i	5	< 0.5	Experiment 2			
	Serine tRNA _{III}	1.3	19.6	<i>E. coli</i> tyrosine tRNA _{II}	0.01	35.6	
	Leucine tRNA _{II}	2.5	15.1	poly(A)	0.1	2.3	
	Tyrosine tRNA _i	0.65	38.8	poly(C)	0.1	0.5	
	Tyrosine tRNA _{II}	0.65	42.6	poly(U)	0.1	1.3	
				poly(G)	0.1	1.3	

The standard reaction mixture contained in a total volume of 0.25 ml, 22 μmol of Tris-HCl, pH 8.0, 3.2 μmol of MgCl₂, 0.02 μmol of 2-mercaptoethanol, 0.1 μmol of ³H-GTP (2 × 10⁶ c.p.m. per 0.1 μmol). 30 μg (experiment 1) or 25 μg (experiment 2) of enzyme protein and each of the indicated RNAs as a primer were added. After incubation at 36 °C for 20 min, the cold 5% trichloroacetic acid-insoluble materials were collected on membrane filters, and the radioactivity of the dried filters was counted. Male mice (ddY) were inoculated intraperitoneally with 10⁶ cells per mouse of Ehrlich ascites tumour cells, supplied by Dr D. Mizuno (Tokyo University), and after 6 to 8 d, cells were collected and used for preparation of enzyme and RNA. RNAs were extracted from *E. coli* cells, Ehrlich ascites tumour cells, and tissues of mouse and rat by cold sodium dodecyl sulphate-phenol methods¹⁰. tRNA and rRNA fractions were prepared by 5–30% glycerol gradient centrifugation of whole RNA. Ten purified *E. coli* tRNAs were supplied by Dr S. Nishimura (National Cancer Center Research Institute). Qβ phage RNA was prepared as described by Haruna and Spiegelman¹¹. Other phage RNAs were gifts of Dr T. Shiba (Mitsubishi-Kasei Institute of Life Science). TMV RNA was given by Dr Y. Okada (Tokyo University).

found in the RNA-dependent RNA polymerase fraction⁵ of Ehrlich ascites tumour cells, and we have further purified the enzyme about 30-fold (see legend to Table 2). The whole RNA fraction extracted from these cells was active as a primer (Table 1). When the whole RNA was fractionated, tRNA was about four times as active as the whole RNA, while rRNA was almost inactive. To determine whether all the molecular species of tRNA were active as primers the tRNA fraction of Ehrlich ascites tumour cells was further separated by a reversed-phase chromatography, RPC-5 (ref. 6). Figure 1 shows clearly that some specific tRNAs were quite active as primers and others were inactive. The primer activity of tRNA in the fraction 35 of Fig. 1 was seven times that of unfractionated tRNA. Further separation of the tRNAs and ultimately the sequence analysis of each purified species will be necessary to identify the molecular species of the tRNAs which are active as primers.

To obtain information about the common structural characteristics of the primer tRNAs, we tested the primer activity of various RNAs extracted from *Escherichia coli* (Table 1), since the complete nucleotide sequences of about ten *E. coli* tRNAs have been determined^{7,8}. The whole RNA extracted from *E. coli* was active, and tRNA was also quite active but rRNA was almost inactive. Among the ten purified tRNAs of *E. coli*, only leucine tRNA_{II}, serine tRNA_{III}, tyrosine tRNA_i and tyrosine tRNA_{II} were active as primers. It is interesting that all active tRNAs possess the S-region in their secondary structure, and were eluted at the end of neutral DEAE-Sephadex A-50 chromatography⁹. It should be noted that serine tRNA_i, which was inactive as a primer, was eluted at the top region of DEAE-Sephadex A-50 chromatography, although it possesses the S-region. Thus the primer activity of tRNA molecules may be related to their structural properties which determine their positions of elution in DEAE-Sephadex A-50 chromatography.

The enzymatic reaction showed an absolute requirement for primer RNA (Table 2, experiment 1). The reaction was neither inhibited by creatine phosphate and creatine phosphokinase nor by inorganic phosphate, but was inhibited by inorganic pyrophosphate. This suggests that guanosine tri-

phosphate is the actual substrate. The three ribonucleoside triphosphates other than GTP were not required for incorporation of GMP. The enzyme required magnesium or manganese ions, the optimum concentration being 7–14 mM and about 5 mM, respectively, and it also required 2-mercaptoethanol, its optimum concentration being 0.08 mM. *E. coli* tyrosine tRNA_{II} lost all its primer activity after oxidation by periodate or after digestion with RNase T₁. When ribonucleoside triphosphates other than GTP were offered as substrates, no enhancement of incorporation was observed when tRNA was added (Table 2, experiment 2).

Various nucleic acids other than RNAs extracted from Ehrlich ascites tumour and *E. coli* were tested and found to be inactive as primers (Table 1). Moreover, none of these inactive RNAs was inhibitory to the enzyme reaction, as tested with *E. coli* tyrosine tRNA_{II} as the primer.

The radioactive product synthesised by the enzyme with tyrosine tRNA_{II} as a primer had a molecular size of about 4S as determined by glycerol gradient centrifugation. Treatment with alkali and RNases, such as RNase T₂, RNase T₁, RNase A and PDase (venom phosphodiesterase), made the radioactive product acid soluble. The radioactive substances in the hydrolysates as identified by cellulose thin-layer chromatography, Dowex-1 or DEAE-Sephadex A-25 column chromatography were as follows: alkaline hydrolysis, 2'-GMP and 3'-GMP; RNase T₂ treatment, 3'-GMP; RNase T₁ treatment, 3'-GMP and oligonucleotides; RNase A, oligonucleotides with chain lengths of more than two; and PDase treatment, 5'-GMP. The molecular ratio of incorporated GMP to the primer tyrosine tRNA_{II} reached a maximum of 10–15. These preliminary analyses of the products indicated that the radioactivity existed in GMP residues which were attached covalently to the primer tRNA by phosphodiester linkages. Determination of the structure of the radioactive product is now in progress.

The presence of primer RNA in Ehrlich ascites tumour and the absence in normal mouse liver (Table 1) prompted us to search for primer RNAs in other tissues and tumours of mouse. RNAs extracted from mouse spleen and kidney, as well as liver, were inactive as primers while RNAs extracted from tissues of mice infected with Friend virus or transplanted

Table 2 Characterisation of the reaction

Assay condition	³ H-GMP incorporated (pmol)
Experiment 1 Complete	44.8
–RNA	< 0.5
–Enzyme	< 0.5
–Enzyme+heated enzyme	1.2
+ATP, UTP, CTP (0.1 μmol each)	41.9
+Creatine phosphate (1 μmol), creatine phosphokinase (10 μg)	44.7
+Trypsin (20 μg)	10.0
+RNase A (10 μg)	< 0.5
+DNase (10 μg)	44.6
+Inorganic phosphate (1 mM)	51.8
(10 mM)	49.8
+Inorganic pyrophosphate (1 mM)	48.4
(4 mM)	29.6
(8 mM)	19.3
Experiment 2	(μg)
³ H-GTP+tyrosine tRNA ₁₁	1.3
+ glutamic acid tRNA ₁₁	2.5
³ H-ATP+tyrosine tRNA ₁₁	2.5
+ glutamic acid tRNA ₁₁	2.5
³ H-UTP+tyrosine tRNA ₁₁	2.5
+ glutamic acid tRNA ₁₁	2.5
³ H-CTP+tyrosine tRNA ₁₁	2.5
+ glutamic acid tRNA ₁₁	2.5

Heated enzyme was prepared by heating at 60 °C for 10 min. The complete reaction mixture was as described in the legend to Table 1, except that 30 μg of enzyme protein and 0.33 μg of purified *Escherichia coli* tyrosine tRNA₁₁ as a primer were added (experiment 1). The reaction mixtures in experiment 2 were similar to those in experiment 1 with these alterations: 0.1 μmol each of tritiated ribonucleoside triphosphates (2×10^6 c.p.m. per 0.1 μmol) was used as substrate; the amount of enzyme was 11 μg, and purified *E. coli* tRNA was added as a primer. In experiment 2, the radioactivity for the sample without added tRNA was subtracted from each value. Washed Ehrlich ascites tumour cells were suspended in lysis buffer (0.1 M Tris-HCl, pH 7.4, 0.01 M MgCl₂, and 0.0005 M 2-mercaptoethanol) containing DNase (5 μg ml⁻¹), and frozen and thawed once. After homogenisation for 40 s, the mixture was stirred at 4 °C for 30 min and EDTA was added to a final concentration of 0.01 M. The homogenate was then centrifuged at 105,000g for 2 h. From the supernatant the 35–50% (NH₄)₂SO₄ precipitate was collected and dissolved in lysis buffer, and the appropriate amount of protamine sulphate was added to remove nucleic acids. The 60% (NH₄)₂SO₄ precipitate was collected and dissolved in the standard buffer (0.01 M Tris-HCl, pH 7.4, 0.005 M MgCl₂, 0.0005 M 2-mercaptoethanol and 30% (w/v) glycerol). Remaining (NH₄)₂SO₄ was removed from the dissolved precipitate by passage through a column of Sephadex G-25, previously equilibrated with buffer (0.05 M Tris-HCl, pH 7.4, 0.001 M EDTA, 0.001 M 2-mercaptoethanol and 30% (w/v) glycerol). The flow-through fraction was applied to a phosphocellulose column (P11 Whatman), previously equilibrated with buffer. Elution was started with the equilibrating buffer containing 0.02 M NaCl, and then the concentration of NaCl was changed to 0.2 M. The 0.02–0.2 M NaCl fractions were pooled. The 60% (NH₄)₂SO₄ precipitate was collected, dissolved in the standard buffer and used as enzyme.

Table 3 Survey of primer RNA from mouse

Source of RNA	³ H-GMP incorporated (pmol)
Ehrlich ascites tumour	16.4
Spleen (normal)	1.5
(with Friend virus-induced solid tumour)	20.8
Liver (normal)	2.5
(Friend virus-infected)	15.6
(with Friend virus-induced solid tumour)	13.2
Kidney (normal)	3.2
Friend virus-induced ascites tumour	21.8
Sarcoma 180	19.0

Assay conditions were as described in the legend to Table 1. Enzyme added was 30 μg, and 20 μg of each RNA was added as a primer. RNAs were extracted from spleen and liver of mice infected with Friend leukaemia virus or transplanted with Friend virus-induced solid tumour and from Friend virus-induced ascites tumour, as described previously^{12,13}. Both the solid and ascites strains of Friend tumours were infected persistently with Friend virus¹⁴. Mice with sarcoma 180 were supplied by Kyowa Hakko Co., Ltd.

with Friend virus-induced solid tumour and RNAs from Friend virus-induced ascites tumour and sarcoma 180 were active (Table 3). Though the biological significance of this enzyme remains obscure, these results suggest that, in mouse, primer RNAs (probably specific tRNAs) are specific to tumours or tissues infected with tumour viruses.

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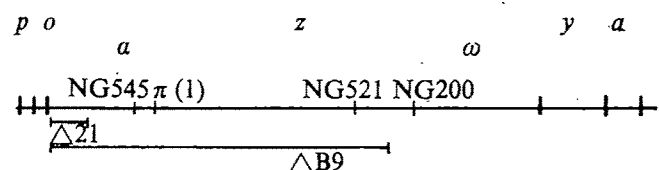
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Mutation blocking the specific degradation of reinitiation polypeptides in *E. coli*

A NONSENSE mutation in a bacterial gene leads to the synthesis of two kinds of incomplete polypeptides^{1–4}. These are the termination, or T, fragments extending from the normal N terminus to the site of mutation, and the reinitiation, or R, fragments initiating distal to the end of the T fragment and continuing to the normal C terminus. Both the T and the R fragments produced by nonsense mutations in the β-galactosidase gene of *Escherichia coli* (z gene) are rapidly and selectively degraded *in vivo*^{5–7}. This contrasts strongly with the wild-type β-galactosidase, which is stable^{5,7}. The degradation of mutant polypeptides implies the existence of proteolytic systems which can distinguish these unstable proteins from the stable ones.

One of the approaches our laboratory has taken to the study

Fig. 1 Genetic map of the *lac* operon of *E. coli*. p, Promoter; o, operator; z, the structural gene for β-galactosidase; y, the structural gene for galactoside permease; a, the structural gene for thiogalactoside transacetylase. The lines below indicate the extent of various deletions. Terminating mutations and π(1), the mutationally generated reinitiation site¹, are shown above the line. α and ω regions are only approximately defined by complementation. All of these mutants have been described^{9,10}. *degR* was isolated from a *recA*[–] diploid between deletion B9 and NG200. B9 ends before the operator but covers all known z[–] mutations at the operator end. This is a new observation about B9, as a result of finer mapping than in the original report¹¹.



of these degradation systems is to isolate mutations that lead to the stabilisation of T and R fragments. The first such mutation was isolated by a selective strategy based on intracistronic complementation⁸. The resulting mutation, originally named *deg* but now called *degT*, stabilises T fragments. We present results in this paper which show that *degT* mutations do not stabilise R fragments. This observation implies the existence of another degradation system which recognises R fragments. By modifying the original selection procedure we have been able to isolate a mutation, called *degR*, which stabilises R fragments against degradation but has little effect on T fragments.

To isolate mutants stabilising R fragments, a *recA*⁻ *F'* *lac* heterogenote containing two *z*⁻ mutations was constructed (see Fig. 1). These *z*⁻ mutations were picked to have such a low level of complementation that the diploid was *lac*⁻ on MacConkey *lac* indicator plates. This lack of significant complementation could be attributed to the rapid rate of degradation of the R fragment, since the T fragment was chosen for its relatively slow decay rate. The diploid was *rec*⁻ to prevent *lac*⁺ bacteria from arising by recombination. This *lac*⁻ diploid was allowed to revert, and on analysis some of the *lac*⁺ revertants were found to contain mutations affecting the degradation of the R fragment. The screening techniques were the same as previously used for *degT* (ref. 8). One of these *lac*⁺ revertants, *degR*₁, was studied extensively.

The effect of *degT* and *degR* on T fragments is shown in Fig. 2. The rate of decay of the NG521 nonsense T fragment is assayed by auto- α complementation¹². Clearly, the *degT* but not the *degR* stabilises this polypeptide. The effect of the

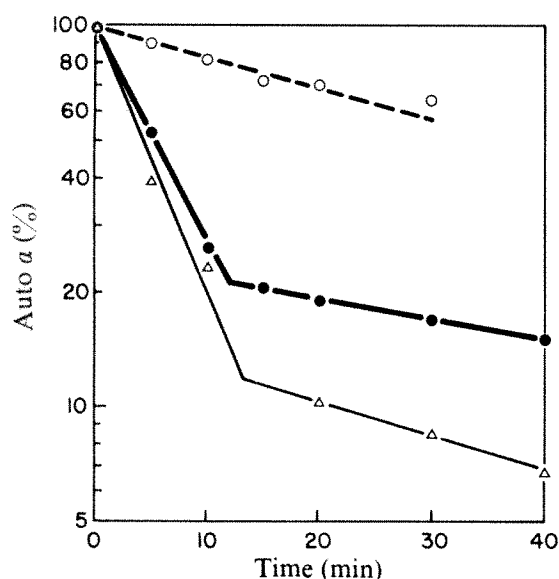


Fig. 2 Kinetics of degradation of a UGA termination fragment in *degT*, *deg*⁺, and *degR* strains. The degradation of termination fragments in exponentially growing cultures after removal of inducer was followed by assay of auto- α donor activity. The cells were grown in 100 ml of standard LB at 37 °C on a shaker, and during exponential growth IPTG (isopropyl- β -D-thiogalactopyranoside) was added to a final concentration of 5×10^{-4} M. After 15 min of induction, cells were centrifuged, washed twice with an equal volume of chilled LB, resuspended in warm LB; and the incubation was continued on a shaker. Samples (10 ml) were withdrawn at regular intervals before and after the removal of IPTG. The auto- α activity of the samples was measured as described by Morrison and Zipser¹². The β -acceptor extract was prepared from a strain carrying deletion 21 in the *z* gene. The amount of auto- α activity present immediately after the removal of IPTG was called 100%, and the percentage of auto- α activity remaining at later times was calculated accordingly. ○, auto- α of 521 (UGA) fragments in *degT* cells; ●, auto- α of 521 (UGA) in wild type cells; △, auto- α of 521 (UGA) in *degR* cells. The genotype of the strain is *F* *pro*⁺ *lac* *z*⁻ 521/*z*⁻ 521, with the exception of the *deg* marker.

two kinds of *deg* mutations on R fragments is shown in Figs 3 and 4. In one case ω complementation is used to follow decay, and in the other the ¹⁴C-labelled polypeptide is visualised by autoradiography after SDS-polyacrylamide gel electrophoresis. *degR* is seen to stabilise both the complementing activity and the physical structure of R fragments, whereas *degT* has no effect. This clear separation of the effects of *degR* and *degT* is typical. Occasionally there is some cross effect of *degR* on T fragments or *degT* on R fragments (Apte, B. N., and Zipser, D., unpublished). We believe this is not a direct effect but the result of stabilising interactions between T and R fragments themselves. Thus, for example, when a T fragment is stabilised by a *degT* mutation it can then interact with an

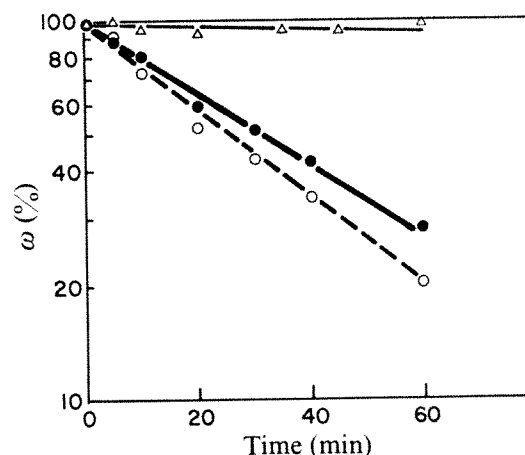


Fig. 3 Kinetics of degradation of ω complementing activity in *degT*, *deg*⁺, and *degR* strains. The cells were grown in 200 ml of standard LB at 37 °C on a shaker, and during exponential growth IPTG was added to a final concentration of 5×10^{-4} M. After 30 min of induction, cells were centrifuged, washed twice with an equal volume of chilled LB, and resuspended in warm LB; and incubation was continued on a shaker. Samples (20 ml) were withdrawn at regular intervals before and after the removal of IPTG. ω complementing activity was measured as described by Ullman *et al.*¹⁰ with some modification. The cells were collected by centrifugation; the pellet was suspended in 1 ml of TM buffer (0.01 M Tris, 0.01 M MgCl₂) and sonicated with a Biosonik IV sonicator. The sonicates were centrifuged, and 0.5 ml of the supernatant was mixed with 0.5 ml of ω acceptor extract from the *z*⁻ NG200 mutant. The mixture was incubated at 21 °C for 90 min and an aliquot was assayed for β -galactosidase activity. The amount of ω donor activity present immediately after removal of the inducer was called 100%, and the percentage of ω donor activity remaining at later times was calculated accordingly. ○, ω -donor activity in *degT* cells. ●, ω -donor activity in the wild type. △, ω -donor activity in *degR* cells. The genotype of the strain is *F* *pro*⁺ *lac* *z*⁻ 545- π (1)/*lac* *z*⁻ 545- π (1), with the exception of the *deg* marker.

R fragment to slow its degradation. A similar effect would be expected from stabilising R fragments.

The data in Fig. 4 indicate that the half life of the π (1) restart fragment increases from about 5 min in wild type to over 30 min in the *degR* background whereas the half life of the unstable ω complementing activity increases by at least the same factor. The apparent rate of synthesis of π (1) goes up about fourfold, as indicated by the amount of ¹⁴C-leucine incorporated in a 4 min pulse. There is also an increase of about 65% in the amount of β -galactosidase produced when a wild type *z* allele is put in the *degR* background. The cause of this modest increase is not known. It could be explained, however, if some nascent β -galactosidase was degraded by the R system in a wild type but not in the *degR* background.

The simplest interpretation of the data presented here is that *degR* is a mutation affecting some feature of the degradation system for R fragments in *E. coli*.

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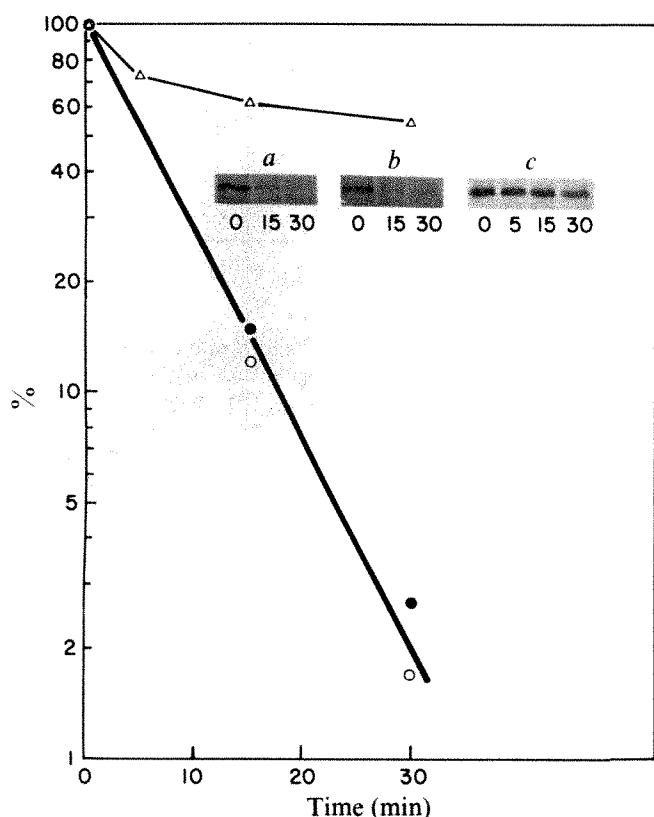


Fig. 4 Kinetics of degradation of reinitiation fragment in *degT*, *deg+*, and *degR* strains. The strains were grown in side-arm flasks containing 80 ml of minimal glycerol medium. The cells, at a density of approximately 5×10^8 cells ml^{-1} , were induced by addition of IPTG to a final concentration of 5×10^{-4} M. After 2 min of induction the culture was labelled for 5 min with $10 \mu\text{Ci}$ of ^{14}C -leucine ($312 \mu\text{Ci} \mu\text{mol}^{-1}$), at which point a 1,000-fold excess of cold leucine was added. Just before the chase and at regular intervals during the chase, 20-ml samples were withdrawn, centrifuged, resuspended in 0.5 ml of TM buffer, and sonicated. The samples were centrifuged, and $5 \mu\text{g}$ of pure β -galactosidase and $15 \mu\text{l}$ of anti- β -galactosidase serum were added to each sample. The samples were allowed to stand at 4°C for 2 h, after which they were centrifuged and the antigen-antibody complex washed three times with TM buffer. The pellet was finally dissolved in $50 \mu\text{l}$ of SDS sample buffer by heating at 100°C for 10 min. Fifteen microlitres of each sample were loaded on 10% SDS-polyacrylamide gel slabs, which were made and run according to Studier¹² with the discontinuous buffer system of Laemmli¹³. The gels were stained, dried, and autoradiographed on Kodak medical X-ray film. The film was scanned with a Joyce-Loebl microdensitometer. The areas under $\pi(1)$ peaks in different samples, representing the amount of radioactive $\pi(1)$ fragment present, were normalised with respect to another stable peak in all samples. The amount of fragment just before the chase was called 100%, and the percentage of radioactive fragment remaining at other times during the chase was calculated accordingly. \circ , Degradation of $\pi(1)$ in *degT* cells; \bullet , degradation of $\pi(1)$ in the wild type; \triangle , degradation of $\pi(1)$ in *degR* cells. A part of the autoradiogram from which the data for the decay curves were obtained is shown in the upper right corner of the figure. *a*, Degradation of $\pi(1)$ in *degT* cells; *b*, degradation of $\pi(1)$ in *deg+* cells; *c*, degradation of $\pi(1)$ in *degR* cells. The time at which the sample was taken is indicated below each sample. The genotype of the strain is $F' \text{ pro}^+ \text{ lac } z^{-545} \cdot \pi(1) / \text{lac } z^{-545} \cdot \pi(1)$, with the exception of the *deg* marker.

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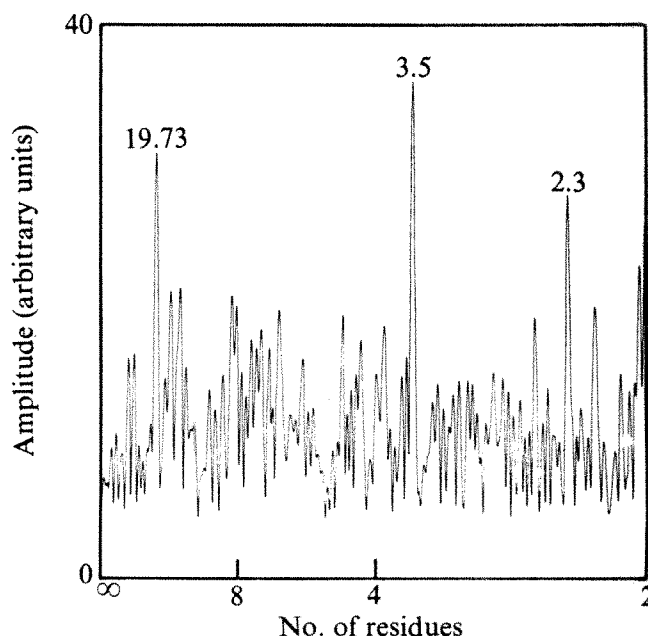
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Fourteen actin-binding sites on tropomyosin?

TROPOMYOSIN plays an important part in the control of muscle contraction. It is a rod-shaped, coiled-coil molecule, about 410 Å long, composed of two parallel α -helical chains which are in register¹⁻⁴. It lies in the grooves of the actin double helix of all known types of muscle filament and is normally thought to be associated with seven actin units⁵⁻⁷. Calcium regulates the contraction of vertebrate skeletal muscle by its influence on troponin, which in turn leads to a movement of tropomyosin in the actin groove⁸⁻¹⁰, thereby exposing (in the 'on' position) or masking (in the 'off' position) the myosin cross-bridge binding areas. The position of the troponin-binding site is known fairly precisely (ref. 11 and review ref. 4), but the actin-binding sites have not yet been identified. Here, we analyse a fourteen-fold periodicity in the amino acid sequence of α -tropomyosin¹² from rabbit skeletal muscle and propose that it is associated with seven pairs of quasi-equivalent actin-binding sites. Parry¹³ and Stone *et al.*¹² first noted several series of amino acid types with a repeat of about $19\frac{1}{2}$ residues, and areas low in acidic residues spaced about 40 residues apart. There is also a slightly irregular 42-residue repeat resulting

Fig. 1 Fourier transform of the acidic residues of tropomyosin. Note the periodicity at 19.73 residues in addition to the second and third orders of the heptapeptide repeat at 3.5 and 2.3 residues. The transform was computed by expressing the chemical sequence¹² as a mathematical sequence of ones (for acidic residues) and zeros (for the rest) and, to enhance the fine detail of the transform, this was made the first 284 terms of a sequence of 2,048, the rest of which were zero. To enable different transforms to be compared directly, the mean of this sequence was adjusted to zero and its root mean square scaled to an arbitrarily chosen value of 10 before transformation.



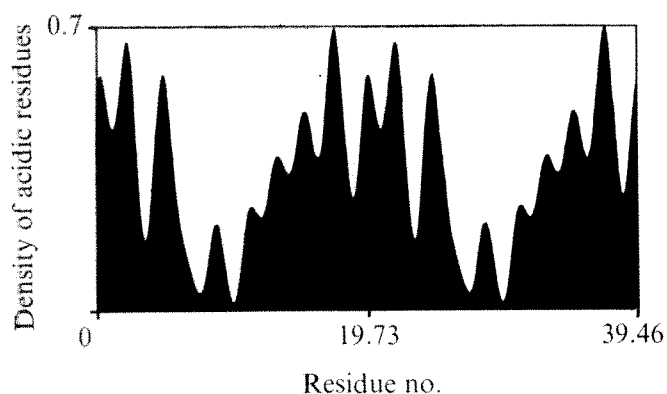


Fig. 2 Average composition of the acidic residue repeat unit (two consecutive repeats shown). The origin corresponds to the N-terminus of the sequence or points multiples of 19.73 residues from it. The distribution was computed by convoluting the mathematical sequence with a series of points spaced 19.73 residues apart. This is equivalent to adding the sequence to itself with staggers of 19.73 residues.

from gene duplication¹⁴ which is in phase with them. We used Fourier analysis to make an objective and systematic search for periodicities in the distributions of acidic, basic and non-polar groups, and here assess their significance.

Figure 1 shows the Fourier transform for the acid groups, which shows a strong periodicity at about 20 residues. By expanding the series to 17,040 terms the peak was fixed at between 19.722 and 19.745 residues, and we take 19.73 as the best estimate. The non-polar and basic distributions showed a much weaker variation 180° out of phase with the acid profile. Table 1 gives their amplitudes and phases. A detailed hand examination confirms that the periodicity persists along the entire sequence. Figure 2 illustrates the average distribution of acid groups in the 19.73-residue period and shows that they are grouped into bands about 10–12 residues wide. Thus our study shows that the periodicity is closer to 19.73 than 19½ residues, and is strongest in the negative charges. There are also marked periodicities at 7/2 and 7/3 residues, which appear because the supercoiling of the helix depends on a heptapeptide repeat pattern in the sequence^{2,12,15} of the type *a.b.c.d.e.f.g*. Residues at positions *a* and *d* are normally non-polar, at *e* often acid, and at *g* basic.

It is important to establish whether the 19.73-residue periodicity could arise by chance. To do this, artificial tropomyosin sequences were constructed by shuffling residues between heptapeptides. Residues in positions of type *a* were shuffled randomly, as were those of type *b*, and so on, producing sequences in which the heptapeptide pattern persisted but other features were randomised. These sequences were then transformed and their amplitude at a frequency of 19.73 residues determined as for the tropomyosin sequence. For the acidic residues the average value of this amplitude for 5,000 such sequences was 8.9 with a standard deviation of 4.6. The maximum value obtained was 29.4 and 99.9% of the values were less than 25.5. Therefore, the value of 31.7 obtained at this frequency for the acidic residues in the real sequence is highly significant. The low value obtained for the basic residues is clearly not significant, but the significance of the value of 20.4 obtained for the non-polar residues is not immediately clear, because the fact that these residues will only be found in the gaps left by the acidic periodicity will tend to produce a periodicity even if they occupy the gaps randomly. To test this point, random sequences were again generated, but only the positions of the basic and non-polar residues were randomised within the heptapeptide structure; the positions of the acidic residues were not changed. The mean value of the amplitude at 19.73 residues for 5,000 such sequences was 18.8 with a standard deviation of 4.3 and 27% of these values exceeded the value of 20.4 obtained with the real sequence. Thus the non-polar repeat may only be a consequence of the

Table 1 Amplitudes and phases of the transforms of the tropomyosin sequence at a frequency of 19.73 residues

	Type of residue		
	Acidic	Basic	Non-polar
Amplitude (arbitrary units)	31.7	12.0	20.4
Phase (degrees)*	13.5	168	–155

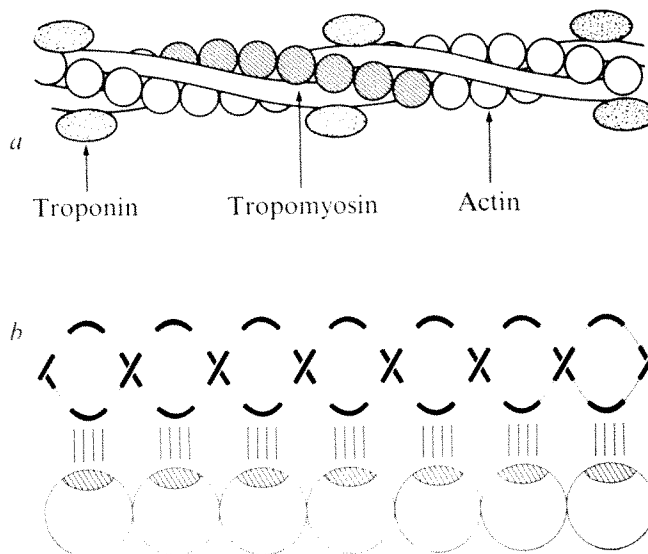
* Referred to a phase origin at the N-terminus of the sequence.

repeat in the acidic residues. A hand examination of the sequence, however, reveals some regularities in the non-polar groups at positions *b*, *c*, *f* of the helix which suggest that these groups are themselves significant.

How is the 19.73-residue length related to the structure of tropomyosin itself, and its interaction with actin? Its chains appear to be in register, so the end-to-end association of tropomyosin is thought to involve an overlap of molecular ends^{3,16}. The 19.73-residue pattern will repeat 14.39 times in 284 residues (the length of the sequence¹²) and so, if the molecules were joined without overlap, the periodicity would be interrupted on passing from one molecule to the next. Observations on paracrystals¹⁰, together with the points mentioned below about the interaction with actin suggest that there should be no interruption. This implies an overlap of about eight residues, when the apparent length of the molecule would be 276 residues, 14 times the repeat distance. If the axial residue length in tropomyosin is taken as 1.49 Å (ref. 17) a sequence of 276 residues would have a length of 411 Å, instead of 423 Å for 284 residues, which would agree well with the value of 410 ± 5 Å found in crystals⁴.

In its interaction with actin, the three-dimensional structure of tropomyosin is important. Longley¹⁸ has proposed that the tropomyosin coiled coil makes seven half turns in a molecular length. We prefer the view¹⁶, endorsed by Parry¹⁹, that there are only six half turns, so that the coiled coil makes seven half

Fig. 3 Scheme for the binding of tropomyosin to actin. *a*, Arrangement of proteins in the thin filament; *b*, interaction of seven actin units with a tropomyosin molecule. This projection corresponds to unwinding the actin helix in (*a*) so that the seven shaded actin units lie in a straight line. This operation preserves the relative positions of the actin and tropomyosin interaction sites but imparts an additional half turn to the tropomyosin coiled coil so that its apparent pitch in this projection is reduced to 114 Å as opposed to 137 Å in the thin filament. The 28 negative zones on the tropomyosin molecule (indicated on the coiled-coil by dense regions) are arranged in four series of seven and one of these series is placed so that each of its zones makes an equivalent contact with an actin unit.



turns relative to the actin subunits when it follows the twist of the actin helix in the thin filament. With the chains in register, the 14 acidic zones on each chain come together to give 28 acidic surface zones on the outside of the tropomyosin supercoil which form 14 diametrically opposed pairs 29.3 Å apart. Each pair of zones has a twist of 90° relative to the next (Fig. 3) so that the 28 zones form four series of seven when viewed along the direction of the actin helix. The zones of one of these series are spaced so that they can interact with seven consecutive actins on one side of the actin groove and it would therefore seem likely that the acid-rich zones represent the regions of the tropomyosin which bind ionically to actin, while the non-polar groups may make additional hydrophobic contacts. The 14 zones probably represent two different series of binding sites, with one set of seven being used in relaxed muscle ('off'), and one or both in the tense state ('on'), when tropomyosin changes its position⁸⁻¹⁰. The sites on adjacent zones may be so placed as to interact with both strands of the actin helix, since the projection of the 29.3 Å zone spacing also matches the axial stagger between actins. The three-dimensional reconstructions of actin-tropomyosin²⁰ seem to be consistent with these proposals.

An analysis (which will be published elsewhere) of the staining patterns in magnesium paracrystals indicates that the molecules lie with their acid-rich zones opposite one another, and suggests that magnesium bridges between opposing zones are a major source of stability in these structures. The same type of bridge would be expected to form in actin paracrystals²¹ which also appear with magnesium. As magnesium ions seem to be necessary for the binding of tropomyosin to actin (except in strong salt solutions)^{22,23} we propose that the tropomyosin-actin interaction involves magnesium bridges between zones of negative charge on both molecules, although there may also be direct salt bridges to positive zones on actin. As the charged amino acids have long flexible side chains, both types of linkage could couple residues several Å apart. This could explain the irregularities in the composition of the acidic zones of tropomyosin, since for the linkage to form it would only be necessary for the particular residue on tropomyosin to lie in a general area (probably three or four residues long).

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Oxygen affinity and allosteric effects of embryonic mouse haemoglobins

THE formation of a circulatory system in the ontogeny of mammals coincides with the appearance of nucleated erythroid cells which are produced in the blood islands of the yolk sac. These cells contain specific embryonic haemoglobins (Hbs) which probably transport oxygen during the earliest stages of mammalian development. Such embryonic Hbs have been demonstrated in man¹⁻⁵ and several other mammalian species⁶⁻⁸. Structural investigations of these pigments showed them to be tetramers which contain unique α -type or β -type subunits. It was also shown that the embryonic α -type subunits of man, mouse and rabbit have more sequence similarity among each other than between the embryonic and the adult α subunit of the same species, indicating that the divergence of embryonic and adult α subunits is much older than mammalian evolution⁹.

This fact is contrasted by sparse data on their function. Farooqui and Huehns⁹ have obtained oxygen dissociation curves on nucleated erythroblasts of human embryos which contained more than 30% embryonic Hbs, and found no significant difference compared with the oxygen-binding properties of human foetal red cells. Studies on the oxygen equilibrium and the Bohr effect of pure Hb Portland revealed, however, that the intrinsic oxygen affinity of this human embryonic Hb is higher than that of adult Hb¹⁰. We show here that the oxygen affinity of mouse embryonic Hbs in the presence of physiologically significant allosteric effectors is considerably higher when compared with adult mouse Hb and that Hb Portland and the embryonic mouse Hbs share some important respiratory characteristics.

Erythroid cells obtained from the peripheral blood of 12.5 d-old mouse embryos¹¹ (strain BALB/c) were washed in saline, lysed by sonification at 4 °C, and the haemolysate centrifuged to remove the cell debris. The clear supernatant was then passed over Sephadex G25 columns equilibrated with bicarbonate or *bis*-Tris buffer for oxygen-binding experiments in the presence and absence of CO₂, respectively. When examined by isoelectric focusing electrophoresis or ion-exchange chromatography on CM cellulose, the haemolysate contained three embryonic Hbs: Hb E_I (50%), Hb E_{II} (30%), Hb E_{III} (16%) as well as traces of adult Hb. Hb E_I consists exclusively of embryonic subunits, whereas Hb E_{II} and Hb E_{III} each contain a pair of adult α subunits^{6,8,12}. Oxygen-binding properties of the unfractionated haemolysate were examined spectrophotometrically in a diffusion chamber^{13,14} at 37 °C and the results compared with those obtained with Hb solution from adult mice (BALB/c) which had been purified by repeated passage over a mixed-bed ion-exchange resin, and then on Sephadex G25 columns.

The partial pressure of oxygen at which Hb is half-saturated (P_{50}) was lower in embryonic than in adult Hb (10.5 and 17 mm Hg, respectively; *bis*-Tris buffer, pH 7.2) which means that the intrinsic oxygen affinity of mouse embryonic Hb is higher than that of the adult pigment (Fig. 1), which was also found in Hb Portland¹⁰. In addition, the change in P_{50} with varying pH in the alkaline range was less pronounced in embryonic than in adult mouse Hb ($\Delta \log P_{50} / \Delta \text{pH} = -0.36$ and -0.48 , respectively). Cooperativity of oxygen binding was, however, similar in both pigments when judged from the exponent n in Hill's equation¹⁵ ($2.6 < n < 3.0$). Perhaps the most striking difference between adult and embryonic Hb with respect to the oxygen-linked proton binding is the shift of the so-called reversed Bohr effect towards neutral pH in embryonic Hb (Fig. 1). We interpret this phenomenon biologically as being advantageous for the embryo as a decrease in pH below 7.25—that is, in a severe acidosis—causes no further fall in oxygen affinity and therefore no impairment of its oxygen uptake. Oxygen release from adult Hb on the other hand, is facilitated in such a situation. The same diminished reactivity towards oxygen-linked

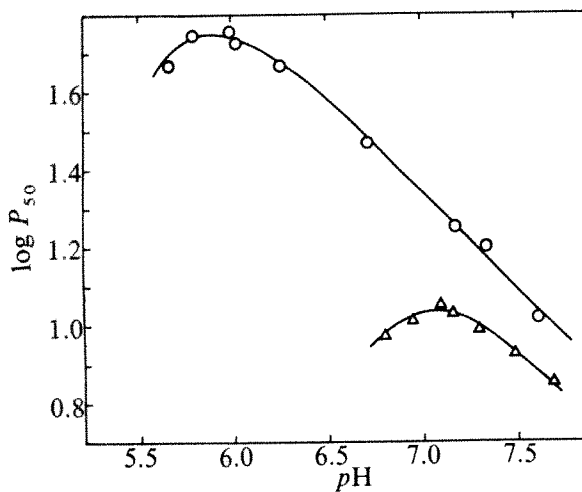
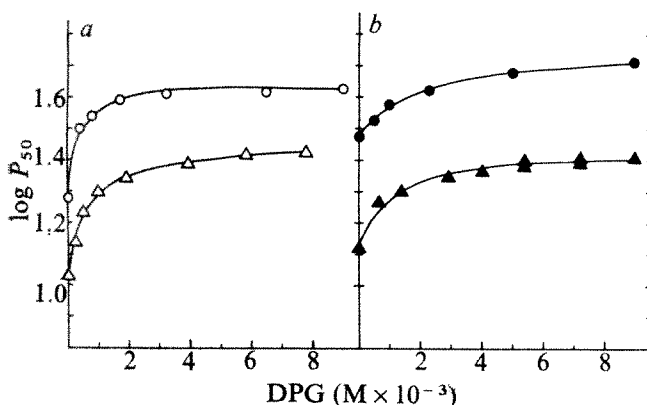


Fig. 1 Plot of $\log P_{50}$ against pH of adult (\circ) and embryonic (Δ) mouse Hb. Temperature, 37°C ; Hb concentration, 0.3×10^{-3} M per 1 tetramer; buffers, 50 mM *bis*-Tris–100 mM NaCl at $pH \leq 7.5$ and 50 mM Tris–100 mM NaCl at $pH > 7.5$. Neither preparation of adult or embryonic Hb samples contained DPG when evaluated by a kinetic test procedure¹⁸.

protons has been observed with Hb Portland, where $\Delta \log P_{50} / \Delta pH$ is only half as large as in adult human Hb¹⁰. A further similarity concerns the reversed Bohr effect which becomes effective at about pH 7 in both types of embryonic Hb and not at pH 6, as it does most mammalian Hbs including human foetal Hb.

Apart from protons, there are at least two more allosteric agents, CO_2 and 2,3-diphosphoglycerate (DPG) which influence the oxygen affinity of Hb. On addition of CO_2 ($P_{\text{CO}_2} = 40$ mm Hg) at pH 7.2, $\log P_{50}$ increased from 1.02 to 1.12 in embryonic and from 1.23 to 1.46 in adult Hb (Fig. 2). This rather weak effect of CO_2 on embryonic mouse Hb suggests that the N-terminal α -amino groups, where oxygen-linked carbamate is being formed¹⁶, are less accessible for CO_2 compared with adult Hb, possibly because the N-terminal amino groups of some subunits are blocked. The maximal effect of DPG on the oxygen affinity of adult and embryonic mouse Hb was about equal when judged from the shift in $\log P_{50}$ produced by DPG (Fig. 2 a). The combined effect of both chemicals was such that at a given concentration of DPG, P_{50} for embryonic Hb was slightly lower in the presence of CO_2 than in its absence, whereas P_{50} of the adult Hb increased even further (Fig. 2 b). It thus became evident that at physiological pH and temperature the high oxygen affinity of embryonic Hbs is maintained in the

Fig. 2 Plot of $\log P_{50}$ at various concentrations of DPG of adult (\circ , \bullet) and embryonic (Δ , \blacktriangle) mouse Hb. a, No CO_2 , b, P_{CO_2} is 40 mm Hg. Temperature, 37°C ; pH 7.2; Hb concentration, 0.3×10^{-3} M per 1 tetramer; buffers, 50 mM *bis*-Tris and 100 mM NaCl in the absence of CO_2 (a), and bicarbonate–NaCl buffer with a molarity of 150 mEq l^{-1} in presence of CO_2 P_{CO_2} 40 mm Hg, (b). DPG used as sodium salt.



presence of allosteric effectors which are likely to be of significance for the control of the oxygen affinity in embryonic blood.

To estimate the influence of pH , CO_2 and DPG on the oxygen affinity of Hb *in vivo*, one needs actual measurements of these variables. As far as pH and P_{CO_2} are concerned it is impossible to collect blood anaerobically from 12.5-d-old mouse embryos in quantities sufficient for the analytical procedures now available. On measuring DPG concentration, which requires less material, in embryonic blood, we found 1.35×10^{-3} M DPG per 1 nucleated erythroid cells compared with 10.8×10^{-3} M DPG per 1 adult erythrocytes. As both Hb and DPG are likely to be present only in the cytoplasm of the erythroid cells one has to correct the former value by the volume taken up by the nucleus. From a large number of measurements of the diameter of the nucleus we estimated its volume to be $250 \mu\text{m}^3$, assuming a spherical shape. Mean cellular volume of the erythroid cells was $470 \mu\text{m}^3$ so that the corrected DPG concentration is 2.9×10^{-3} M DPG l^{-1} . The mean cellular Hb concentration in the cytoplasm of these nucleated erythroid cells is 27%, giving a molar ratio of DPG to Hb tetramer of about 0.7, compared with about 2.0 in the erythrocytes of adult mice. We conclude that the concentration of DPG in erythroid cells is high enough to produce a significant decrease in oxygen affinity.

Our results show that in all experimental conditions investigated, embryonic Hb has a much higher oxygen affinity than the adult form. We conclude that the respiratory function of blood during the very early stages of mammalian ontogeny has characteristics which tend to facilitate embryonic oxygen uptake.

More structural and functional analyses of embryonic Hbs should provide an answer to the question of whether the high oxygen affinity of these respiratory proteins is a selective factor in evolution, as seems to be the case for the salient allosteric effects of adult Hb¹⁷. In favour of such an assumption is the similarity of intrinsic oxygen affinity and Bohr effect of mouse and human embryonic Hbs.

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Conformation of thyroid hormone analogues

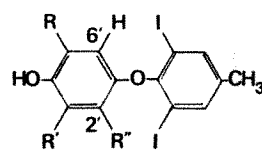
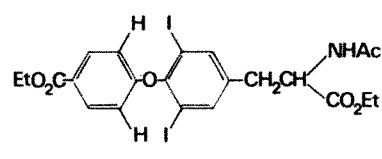
COMPARISON of the activities of conformationally 'fixed' derivatives of thyroid hormones has led to the proposal that the active conformation of 3,5,3'-triiodo-L-thyronine (L-T_3) is one in which the 3'-iodine is distal to the inner (α) ring^{1–4} (Fig. 1). These conclusions have prompted several X-ray studies aimed at determining the conformational preference in the crystals of thyroid hormones^{5–10}. Such studies have shown that the posi-

tioning of the 3'-iodine in the crystal structures of L-T₃ and related compounds is dependent on the crystallising medium, thus suggesting that the distal and proximal forms of L-T₃ are of similar energy. These findings prompted us to investigate the temperature dependence of the proton nuclear magnetic resonance (NMR) spectra of thyroid hormone analogues to determine, in solution, both the magnitude of the barrier to rotation around the ether oxygen and, in unsymmetrical molecules, the relative populations of distal and proximal forms.

Previous work¹¹ on 2,6-diisopropylidiphenyl ethers, together with reported steric parameters for iodine and isopropyl¹², suggested that the coalescence temperature for the 2',6' proton signals of thyroid hormones would be below -60 °C. Since, at this temperature, the thyroid hormones would be practically insoluble in most solvents, we chose, instead, to investigate the model compounds (1-4 in Table 1). The steric and electronic consequences of replacing the alanine side chain by a methyl group are not expected to affect markedly the intrinsic rotational properties of the ether bond⁴. Nevertheless, we recognise that this may alter the dipole moment of the molecule and that this in turn may affect the conformer population in solvents of high dielectric constant¹³.

The 60-MHz spectrum of the thyroxine model compound (1) (Table 1), in tetrahydrofuran, shows a time averaged singlet (7.18 p.p.m.) for the 2',6' protons at 40 °C. On cooling, this

Table 1 Coalescence of 2',6' protons of thyroid hormone analogues

			
(1)	R = R' = I, R'' = H	(5)	
(2)	R = H, R' = I, R'' = H		
(3)	R = R' = R'' = H		
(4)	R = R' = H, R'' = Me		
Compound	T _c (°C)	Δν (Hz)	ΔG _c [‡] (kcalorie mol ⁻¹)*
(1)	-94 ± 3	76.5	8.5 ± 0.2
(2)	-105 ± 4	73 (2'-H)	7.9 ± 0.3
		68 (6'-H)	
(3)	< -110 ± 2	—	< 7.7
(5)	-79 ± 3	65	9.3 ± 0.2

* 4.18 J = 1 calorie

Spectra were recorded on a Varian A60A (60 MHz) spectrometer in sodium dried tetrahydrofuran. Estimated errors in ΔG were based almost exclusively on uncertainties in T_c.

singlet broadens and eventually separates into two broad peaks (6.57 and 7.84 p.p.m.) (Fig. 2). At the coalescence temperature (-94 °C) a rotational barrier (ΔG₁₇₉[‡]) of 8.5 ± 0.2 kcalorie mol⁻¹ was calculated (Table 1) from the Eyring Equation¹⁴.

At 40 °C, the spectrum of a solution of the T₃ model compound (2) in tetrahydrofuran shows a typical ABC pattern for

Fig. 1 Distal (a) and proximal (b) conformations of T₃ analogues.

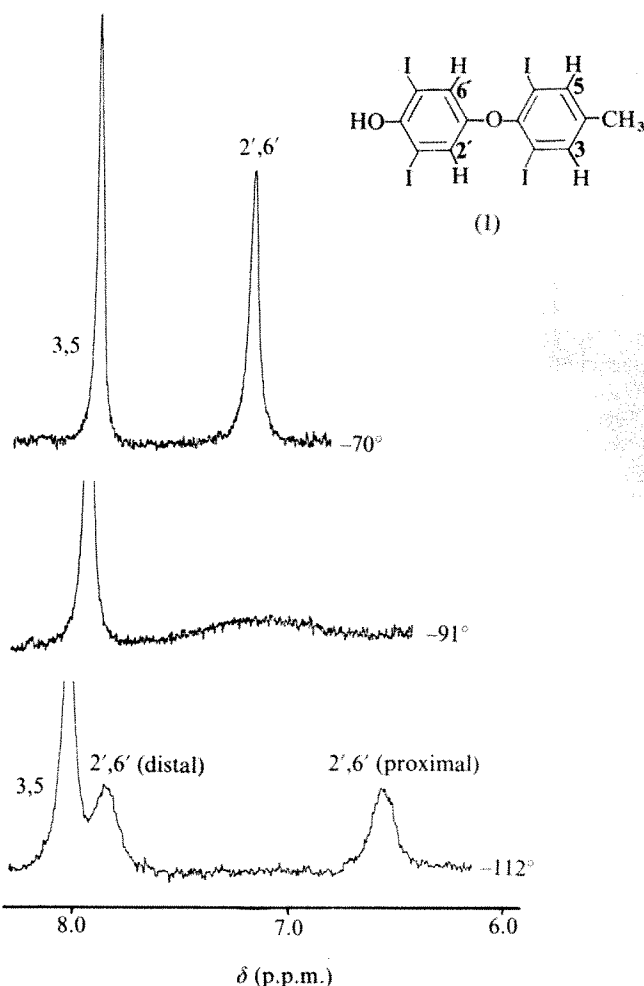
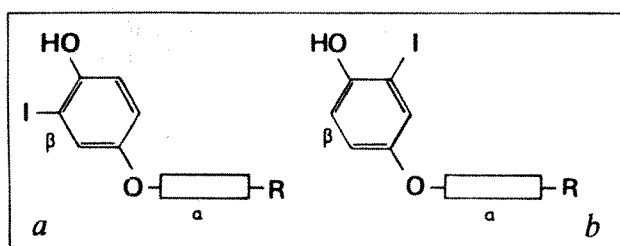


Fig. 2 Variable temperature NMR spectra of compound (1).

the 2', 5' and 6' protons (Fig. 3). The spectrum was analysed with the aid of a simple ABC computer program which provided accurate chemical shifts¹⁵. On cooling the solution to -108 °C, at which temperature rotation about the C(1')-O bond was slow on the NMR time scale, we expected to see the original ABC pattern replaced by two overlapping ABC patterns. Only signals for the individual 2'-protons in each of these two ABC patterns can be easily distinguished, however. Thus the 2' proton (7.1 p.p.m.) separates into two broad single peaks (6.5 and 7.72 p.p.m.) of equal intensity (Fig. 3). At the coalescence temperature (-105 °C) a rotational barrier (ΔG₁₆₈[‡]) of 7.9 ± 0.3 kcalorie mol⁻¹ was calculated (Table 1). At -108 °C the 6' proton separates into two broad peaks with approximate shifts of 6.1 and 7.3 p.p.m. (Fig. 3). The T₂ derivative (3) showed only exchange broadening of the 2',6' proton signals at the solvent limit of -110 °C, indicative of a barrier to rotation (ΔG₁₆₃[‡]) of less than 7.7 kcalorie mol⁻¹.

It is apparent from these results that rotation around the ether bond is governed by non-bonding interactions between the 2',6' hydrogens and 2,6 iodines. If it is assumed that the entropies of activation (ΔS[‡]) for rotations in compounds (1) and (3) are small^{14,16,17} or similar¹⁸ then comparison of ΔG_c[‡] (Table 1) for these two compounds suggests a slightly larger value for the T₄ derivative (1). This difference, if real, is probably caused by the buttressing effect of the 3',5' iodines on the 2',6' hydrogens, an effect previously observed in substituted biphenyls¹⁸. An indication that electronic factors may also affect rotation, as in other systems¹⁷, is suggested by the higher barrier to rotation (ΔG₁₉₄[‡] = 9.3 ± 0.2 kcalorie mol⁻¹) found for the diester (5, Table 1) compared with that of the phenol (3), assuming ΔS[‡] is similar for these two derivatives.

The 2' proton signals in the low temperature (-108 °C)

spectrum of the T_3 model compound (2) were of approximately equal intensity (Fig. 3), indicating an almost equal population of distal and proximal conformations. This represents the first experimental evidence indicating the existence of two conformations of T_3 or one of its analogues in solution. In extrapolating these low temperature results to biological temperature, it was necessary first to determine the variation of the weighted average chemical shifts of the 2' and 6' protons of (2) with temperature in the absence of changes in conformer population. Graphical plots showed that all protons in compounds (1-4) suffered chemical shift changes due to the influence of a common solvent effect. The results for compounds (3) and (4) are of particular interest since they involve only one conformation. The chemical shift of the proximally positioned¹⁹, 6' proton of the 2'-methyl derivative (4) showed a shift towards low field of only 5 Hz over the range +40 to -110 °C. The average chemical shift of the 2' and 6'-protons of (3) showed a similar change. We can conclude from these results that both proximal (2',6') and distal (2',6') protons show conformationally independent chemical shift changes with temperature, but these changes are likely to be (a) small compared with the chemical shift difference between 2' (or 6') proximal and distal protons and (b) similar for proximal and distal protons, that is, the chemical shift difference ($\Delta\nu$) is largely temperature independent. These results enable us to calculate distal-proximal ratios at different temperatures from the equation

$$n_D = \frac{(\nu_{W2'H} - \nu_{W6'H}) + (\nu_{D6'H} - \nu_{P2'H})}{(\nu_{D6'H} - \nu_{P6'H}) + (\nu_{D2'H} - \nu_{P2'H})} \quad (1)$$

where n_D = mole fraction of distal form, and ν_D , ν_P and ν_W refer respectively to distal, proximal and weighted average

chemical shifts. ν_D and ν_P were obtained from the single spectrum of (2) at -108 °C (Fig. 3) and n_D is calculated at any higher temperature by measuring $\nu_{W2'H} - \nu_{W6'H}$ at that temperature. On substituting $\nu_{W2'H} = 427.3$, $\nu_{W6'H} = 393.7$, $\nu_{D6'H} = 435$, $\nu_{P6'H} = 367$, $\nu_{D2'H} = 463$ and $\nu_{P2'H} = 390$ Hz into the above equation, n_D (40 °C) is calculated to be 0.56 (range 0.5-0.6 after taking account of experimental error); this indicates that there is very little change in the conformer ratio over the temperature range studied.

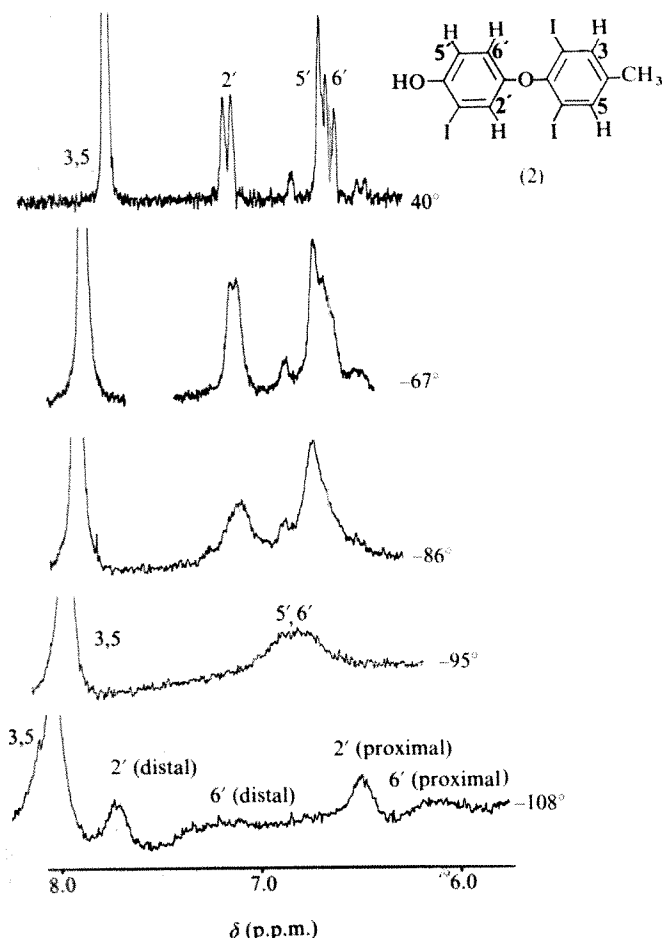
In summary, these results indicate that the barrier to rotation around the ether oxygen in thyroid hormones is in the range 7-9 kcalorie mol⁻¹, in reasonable agreement with a calculated⁴ barrier (11 kcalorie mol⁻¹) for T_3 . Furthermore, the observation of approximately equal populations of distal and proximal forms of the T_3 analogue (2) is not incompatible with an 'active' distal form, as suggested previously^{2,3}.

A recent communication by Camerman *et al.*²⁰ reported the observation of a single species of 3,5,3'-triiodothyropropionic acid in a mixture of diethanolamine, urea and methanol, but claimed to have shown the presence of two non-interconvertible conformations in a 2:1 mixture of ethanol-1 N HCl at room temperature. We believe that this work is in error as, first, the reported shift difference (1.7 Hz) between the 2' proximal and distal protons is negligible compared with those observed by us and other workers¹¹ and, second, the barrier to rotation, reported to be in excess of 20 kcalorie mol⁻¹, is far higher than expected. These apparently anomalous results are readily explained, however, if one assumes partial esterification of the acid by the acidic ethanol used as solvent.

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Fig. 3 Variable temperature NMR spectra of compound (2).



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Errata

In the article "Eccentricity-specific dissociation of visual functions in patients with lesions of the central visual pathways" by E. Pöppel *et al.* (*Nature*, **256**, 489; 1975) Fig. 1 and its legend should have been deleted and do not refer to the paper as published.

The title of the article (*Nature*, **256**, 750; 1975) by B. Donzel *et al.* should have read "Synthesis and conformations of hypothalamic hormone releasing factors: two TRF-analogues containing backbone N-methyl groups" and not as printed.

matters arising

February–June weather relationships in Norway

GREEN¹ claims that the empirical prediction technique can be useful in the development of long range weather forecasting. Empirical methods of long range forecasting sometimes follow the discovery of relationships between observations made at different times and places. Though I agree with Green that there seems to be no reason not to use the relationships in prediction, even though the physical mechanism behind them is not fully understood, some precautions must, nonetheless, be taken. If empirical correlations are to be of any value in weather forecasting, correlation coefficients ought to be considerably larger than the significance levels.

Green illustrated his idea using as an example the correlation between February and June temperatures at Dalen in Norway, between 1940 and 1974. He claims that a cold February will be followed by a warm June. Figure 1, however, shows that points are quite scattered.

An analysis of observed winter and summer temperatures in Scandinavia indicates that no correlation exists that can be useful for a forecast model. Fairbridge² has given differences between July and preceding January temperatures in Stockholm from about 1750. The data indicate a decline in amplitude during the nineteenth century, but a marked increase in recent years.

Table 1 shows the results of a correlation analysis of monthly temperatures at Dalen in Norway. The significance limit is based on a confidence

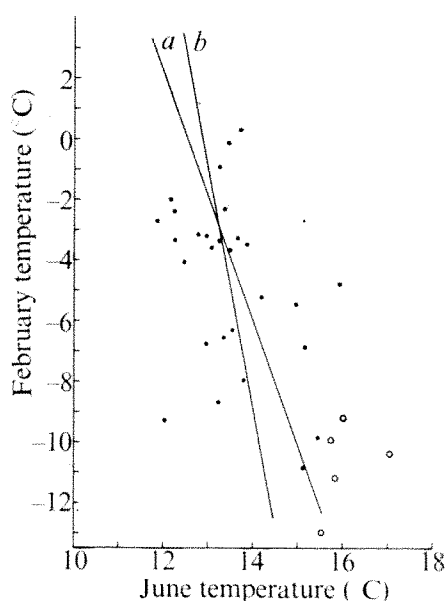


Fig. 1 Relationship between February and June temperatures at Dalen, Norway between 1940 and 1972. *a*, Computed from February–June temperatures for the period 1940–72; *b*, based on the same temperatures except those indicated by circles, showing that the negative correlation depends strongly on a few extreme temperature conditions.

level equal to 95%, in the Student's *t* distribution, and the coefficient is given only when it exceeds the significance limit. For the complete series, 1890–1972, there is no significant correlation between the February–June temperatures. Only by considering a shorter period back from 1972, can a significant correlation be found. Nor is the correlation between the lowest and the highest monthly temperature in the year significant. Similar results have been found in Oslo and Hellisøy.

Table 1 Empirical correlation coefficients for monthly temperatures at Dalen, Norway, between 1890 and 1972

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
January	1.00	0.52	+	+	—	—	+	+	+	+	—	—
February		1.00	0.30	0.30	—	—	+	+	+	+	+	—
March			1.00	0.48	—	—	+	+	+	+	+	+
April				1.00	0.39	—	+	+	+	+	+	+
May					1.00	—	+	+	+	+	+	+
June						1.00	—	+	0.23	+	—	+
July							1.00	0.42	0.22	+	+	—
August								1.00	0.36	+	+	+
September									1.00	0.29	+	—
October										1.00	+	—
November											1.00	0.37
December												1.00

* Significance limit $r_s = 0.22$.

In Britain³ and Norway, the monthly rainfall and temperature are correlated positively in winter and negatively in summer. If a negative correlation between February and June temperatures exists, a positive correlation between February temperature and June rainfall can be expected. An analysis of June rainfall and the temperature of the preceding months for three different places, Oslo, Dalen and Bergen reveals no significant correlation except for February temperatures in Bergen, in which the empirical correlation coefficient is just on the significance level.

I conclude that there is no statistical evidence that the winter temperature is a useful element in forecasting the summer temperature and rainfall for the same domain.

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¹ Green, F. H. W., *Nature*, **253**, 522–523 (1975).

² Fairbridge, R. W., *Encyclopedia of Atmospheric Sciences and Astrogeology*, 205–211 (1967).

³ Murray, R., *Met. Mag., Lond.*, **96**, 141–145 (1968).

Development of visual acuity and the sensitive period

WE have read with interest the article, by Freeman and Marg¹. Their observations that there is a significant change in the response of the visual system of the kitten to spatial gratings with age are not unexpected from the results of our study of the optical properties of kitten's eyes. We question, however, their contention that, on the basis of their ophthalmoscopic observations, "it is unlikely that optical factors limit the acuity determined for younger animals." As evidence to the contrary, we submit the following.

Photographs of the fundus of the same kitten 11, 18, 25 and 32 d after birth are shown in Fig. 1. These photographs were made with a Zeiss fundus camera and accurately demonstrate the hazy media one encounters in kittens for the first 5 weeks of life. This haziness results from the scattering of light by the cornea which is not optically clear in the first 3–4 weeks of life, the tunica vasculosa lentis and by particulate material in the vitreous, much as is seen in premature infants (A. McCormack, personal communication). The tunica vasculosa lentis disappears between days 30 and 35 of life and not before day 23 as contended by Freeman and Marg. To quantify the

degradation of an image by the media of the eye, we have made densitometric measurements of a fluorescein-filled vessel in the posterior pole of the eye. Between days 25 and 32, there was a 43% decrease in the spread of the image of the vessel in the absence of any change in the refractive power or the axial length of the eye. These observations demonstrate that the optical quality of the kitten's eye is improving over this period and must play a significant part in determining the acuity of kittens.

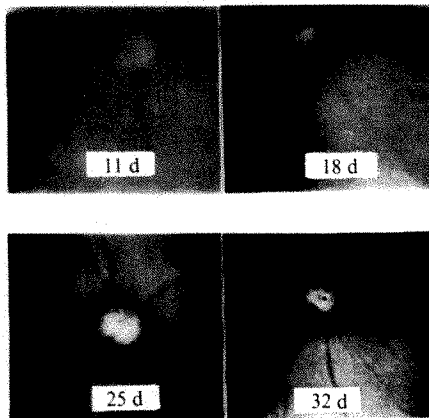


Fig. 1

Further, there is another important factor about the kitten's eye which must be considered by the visual physiologist. We have determined, by retinoscopy, the refractive error of eight eyes from seven kittens. During the first eight weeks of life, with the accommodation paralysed by atropine, the mean refractive error is 7.62 D (range +15.00 D to +4.00 D). It should be noted that these are refractive error determinations for an object at infinity, and any object placed closer will require the kitten to use its accommodative power, which may be limited by the anterior displacement of the lens and its envelopment by the tunica vasculosa lentis. Thus, for example, if the kitten with a 7.50 D hyperopia were placed in the middle of a 50-cm diameter striped cylinder, it would be required to employ 11.5 D of accommodation to bring the stripes into focus. For the reasons cited above, we do not believe the kitten possesses anywhere near this accommodative power. It is indeed surprising that, in spite of these optical defects, it is possible to alter the development of receptive field properties of cortical cells², although these results have not been confirmed (M. P. Stryker and H. Sherk, unpublished). For an out-of-focus optical system with a vertical slit pupil, vertical objects will be in better focus than horizontal objects. If the kitten's visual system responds as does the human astigmatic eye³, the question arises, why are there not more vertically tuned cortical cells in normally

reared cats?

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- ¹ Freeman, R. D., and Marg, E., *Nature*, **254**, 614 (1975).
² Blakemore, C., and Cooper, G. F., *Nature*, **288**, 477-478 (1970).
³ Mitchell, D. E., Freeman, R. D., Millodot, M., and Hagerstrom, G., *Vision Res.*, **13**, 535-558 (1973).

FREEMAN AND MARG REPLY—Accommodation and refractive error has nothing to do with our study as has been suggested¹. The kittens were under general anaesthesia and the eye and its protective contact lens were optically corrected for the 57-cm screen distance.

Optics has a great deal to do with the development of kittens and the determination of their sensitive or critical period of development. It is by means of slits, bars, and edges that the concepts of visual development and deprivation have been uncovered. The original work of Hubel and Wiesel² demonstrated orientation of cortical receptive fields in the kitten as young as 8 d, and others have done similar experiments. The optics of these eyes must be transmitting those forms.

There is no question that the optics of the kitten's eye improves up to about the fifth week, but this does not mean that it is the limiting factor in acuity. With good illumination (rather than a fluorescent source) retinal vessels of about 0.5° width can be resolved at 3 weeks of age. It is likely that the optics are transmitting to the retina gratings of similar detail.

While we await the results of systematic optical modulation transfer function data from the kitten, we stand by our original statement:

"It is clear that the optical quality of the eye of the very young kitten is inferior to that of the adult. But on the basis of ophthalmoscopic observation we feel that it is unlikely that optical factors limit the acuity determined for the younger animals."

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- ¹ Flynn, J. T., Hamasaki, D. I., Flynn, T. E., and Barricks, M., *Nature*, **257**, 337-338 (1975).
² Hubel, D. H., and Wiesel, T. N., *J. Neurophysiol.*, **26**, 994-1002 (1963).

Basalt from DSDP holes

ALTHOUGH Hammond *et al.*¹ are concerned mainly with palaeolatitudes of the Ontong Java Plateau, where DSDP 289 was drilled, they give considerable attention to the basalt at the bottom of the hole. At long last it is good to see another radiometric age for basalt, no matter how flawed the age may seem to the authors. The latter "assign" an age of 109 Myr to

the basalt on the assumption that it must be older than sediments above it which are dated as 108 Myr on biostratigraphic grounds. They find that the radiometric age of 80 Myr which they actually obtain is in "error", and they cite a similar error in a radiometric analysis performed by Cox and Dalrymple² to conclude that the "estimated analytical standard deviation arises almost entirely from uncertainties in the potassium measurements".

I can only suggest that uncertainties cut both ways. If a basalt dated at 80 Myr is to be corrected to 109 Myr it could just as well be corrected in the opposing direction to 51 Myr. In the latter case the basalt would be a sill. Needless to say the basalt would also be a sill if the authors were willing to accept their own date. As to their citation of Dalrymple and Cox I myself would cite MacDougall³. He found a basalt below Campanian strata in DSDP 10, western Atlantic, that gave a radiometric age of 16 Myr. The result was acceptable to MacDougall, from which one concludes that the basalt of DSDP 10 is a Miocene sill intruded into Campanian strata.

Those are the facts; however, impressions may not be out of place. The authors give me the impression that they are "bound and determined" to have the basalt as the oldest material in DSDP Hole 289. Why? Almost certainly because they assume that the basalt is basement. That is a common assumption in modern tectonophysics, but in my opinion it needs to be refuted vigorously. The fact that a few fragments of basalt are the oldest material in a particular hole has no connection with its crustal nature. If the basalt in DSDP were 218 Myr old instead of the assessed 109 Myr old there could still be 1,000 m of little-disturbed strata below. In short, the age of the ocean basins is a problem as wide open now as it was when DSDP drilling began.

In the last analysis my observations are addressed not so much to Hammond *et al.* in particular as to shipboard scientists in general. The scientists seem to go to sea convinced and/or indoctrinated that every piece of basalt is not only the bottom of the stratal sequence but also the bottom of the world. I have no proof to offer that their conviction and/or their indoctrination is incorrect. I submit only that if we are to be comparatively scientific about the nature of the ocean basins we had better drill some 20,000 m of basalts in suitably distributed DSDP holes. Only then could there be a geological dialogue of substance with respect to some of the matters indicated here.

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- ¹ Hammond, S. R., *et al.*, *Nature*, **255**, 46 (1975).
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reviews

THE discovery of X rays by Röntgen in 1895 gave rise to considerable speculation as to whether this radiation was corpuscular, like electrons, or undulatory, like light. As the experimental evidence grew so it became apparent that, like light, X rays must be a form of electromagnetic radiation; Barkla demonstrated that X rays could be polarised and in accordance with J. J. Thomson's theory, this was taken as clear evidence for their being electromagnetic waves; this conclusion was reinforced some years later by Friedrich, Knipping and Laue, who showed that they could be diffracted. Nevertheless there was one property of light which eluded a satisfactory explanation on this wave basis and that was the photoelectric effect. The only simple explanation for this phenomenon was given in 1905 by Einstein, who published his well-known paper on the light quantum interpretation of the photoelectric effect and derived his famous relation $eV = h\nu$. Not much notice was taken of Einstein's contribution and the view was firmly held by the majority of physicists that light and X rays were electromagnetic waves and this therefore excluded them from possessing particle properties. Even as late as 1916 Millikan, in the paper reporting his experiments on the photoelectric effect which exactly confirmed the Einstein relation, commented: "Yet the semicorpuscular theory by which Einstein arrived at his equation seems at present to be wholly untenable". This relationship was accepted as convenient and reliable and it was presumed that it would ultimately prove explicable on electromagnetic theory.

It was at this stage that A. H. Compton came on to the scene. He had started his research under O. W. Richardson at Princeton in 1914 on X-ray diffraction and, after some abortive experiments to look for magnetic scattering of X rays, commenced his experiments on the spectrum of the scattered radiation. These were absorption experiments and they showed that, together with the radiation of unaltered wavelength, there also seemed to be softer radiation present. Barkla had observed this radiation

Waves or particles?

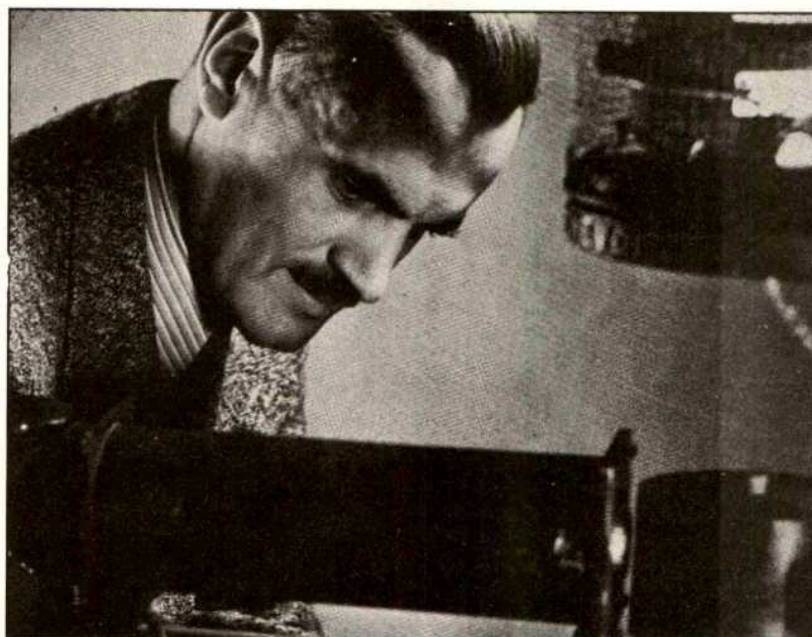


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A. H. Compton at work

and interpreted it as fluorescent radiation characteristic of the scatterer—but longer in wavelength than those lines which can be understood by the Bohr model—and named it J radiation. Compton, adhering strongly to electromagnetic theory, chose to interpret the changed absorption coefficient not as a change in wavelength, but as being due to the size and shape of the electron: by assuming that it was a rather large object and of ring shape, he found that he could account for the reduced absorption coefficient in the conditions of his experiment. After visiting Cambridge in 1919, where Rutherford viewed his large electron with some scepticism, he returned to the US and attacked the scattering problem again, this time using a Bragg spectrometer as a monochromator of the incident X rays. He then found an explanation for the softening of the X rays in terms of Doppler displacement of the radiation from the moving electron, which had received momentum from the incident X rays. He was still, however, unable to account satisfactorily for all the features of scattering and it was only at this stage in October 1922 that he found that a corpuscular interpretation would account fully for all his observations. Quite quickly, most physicists accepted this interpretation of

his convincing experiments and thus accepted the particle nature of electromagnetic radiation. Thus, some 17 years after Einstein's original paper on the photoelectric effect, the scientific community recognised the fact that light and X rays each possessed both wave and particle characteristics.

In this book* Dr Stuewer, an historian of science, traces the ideas and background to Compton's experiments which were to prove important in the development of wave-mechanics. The author, using material drawn from published papers and letters, guides the reader through much of the history of the particle-wave duality. It is liberally referenced and sprinkled with detailed theoretical arguments, and the tenacity with which physicists held to the classical electromagnetic theory emerges very clearly in this book. This piece of physics history will be primarily of interest to physics graduates and, although lacking the gossipy and humorous touches of biography, it nevertheless provides a vivid, enjoyable and definitive account of one of the crucial steps in the establishment of quantum mechanics. It is an unusual work and should have a place in the library of the physicist who is interested in more than the bare bones of his subject.

M. A. Grace

**The Compton Effect: Turning point in Physics*. By Roger H. Stuewer. Pp. xii+367. (Science History: New York, April 1975.) n.p.

Biochemical and biophysical aspects of photosynthesis

Bioenergetics of Photosynthesis. (Cell Biology: A Series of Monographs.) Edited by Govindjee. Pp. xiv+698. (Academic: New York and London, April 1975.) £20.65.

OFTEN books born of long gestation periods are disappointing when they finally arrive. That is definitely not the case with this well edited volume, which should have a very useful life in the library and on the desk.

The book comprises 12 chapters by selected authors who cover the general biochemical and biophysical aspects of photosynthesis in plant and (to a lesser extent) bacterial systems. The way in which this is related to bioenergetic conversion is dealt with from several points of view: membrane structure related to pigments and energy conversion; chloroplast structure; the primary events of photosynthesis including luminescence and fluorescence; oxygen evolution; electron transport; phosphorylation; and ion movements. Each chapter has been externally reviewed and the editor has inserted useful cross references. This, combined with the extensive references, the list of abbreviations and the tables, ensure that each chapter, though complete in itself, is also relevant to other chapters.

The subject index and author index are excellent—necessarily so in a book which claims to “serve as a reference source for researchers but also as an introductory work for graduate students”—and, consequently, the book is more useful than volumes of proceedings and monographs which lack such indexes. It is a pity that more publishers do not insist on the inclusion of extensive indexes even though the resulting books may be more expensive than otherwise—in the long run readers would find the extra expense well worthwhile.

Since the practical use of photosynthesis based on both the improvement of its natural efficiency and the construction of artificial systems are so in vogue now (for food and/or energy) it is a pity that a chapter on this important topic was not included.

An understanding of the biochemical and biophysical mechanisms of photosynthesis seems crucial to its future exploitation and this is where this book will provide the source of background information for present and future investigations—whether scientific, administrative or commercial. **D. O. Hall**

Magnetic oxides

Magnetic Oxides. Edited by D. J. Craik. Part 1: Pp. xxi+482. Part 2: Pp. xix+483-798. (Wiley: London and New York, 1975.) £15.00 each.

THESE two volumes cover various aspects of the theory, properties and uses of magnetic oxides. The 13 chapters are written by 20 authors and cover an extremely wide range, from the practical arts of crystal growing to the theoretical intricacies of crystal fields and exchange. The versatility of magnetic oxides as recording media is also well covered in chapters ranging from the recently utilised bubble domains to the recording over geological times of past terrestrial magnetic fields in the oxides which occur naturally in rocks. There are also chapters on such topics as anisotropy, magnetostriction, optical properties, electrical properties, domains and microwave resonance. Experimental techniques such as neutron diffraction and nuclear magnetic resonance are, of necessity, mentioned here and there but are not discussed fully.

The question of units in a multiple

author book on magnetism is bound to present an initial problem, at least, and it has been solved in this case using e.m.u. throughout, in line with most of the literature. It is, perhaps, not surprising in view of the wide field that the treatment of many of the topics is not detailed enough to be followed by the reader without frequent recourse to references, and in that respect the book tends to be a rather thorough review of the current state of knowledge. That is, however, the major strength of the work and each chapter is well provided with a comprehensive list of references, there being something like 1,700 in the whole book.

Although the individual chapters are to a large extent independent of each other, the work as a whole is well integrated by frequent cross-referencing where interaction of subject matter does occur. The book is perhaps most likely to appeal to the specialist who wishes to extend his knowledge of other aspects of magnetic oxides, and for that purpose it is of considerable value in view of its logical presentation and comprehensive referencing.

A. Stephenson

Kets, bras and boson variables

Spinors in Hilbert Space. By P. A. M. Dirac. Pp. vi+91. (Plenum: New York and London, 1974.) \$14.50.

ON reading this volume, I had at once an impression of *déjà vu*, hearing again the logical, elegant and irresistible exposition so well known to us from Professor Dirac's classic work *The Principles of Quantum Mechanics*. This book differs from the latter, however, in being primarily mathematical in character. Its relevance to the physics of the real world is mentioned in only a few of its 35 sections. The book is based on a series of lectures which the author gave at the University of Miami in 1969. Although self-contained, it is not really intended for the beginner; for example, familiarity with the notion of a spinor and its properties and uses is very much taken for granted, although it is true that four lines in Section 2 do give a complete characterisation for a spinor quantity.

Dirac begins by developing the theory of vectors and operators in a real Hilbert space with $2N$ dimensions, in which there exists the concept of perpendicularity (as opposed to orthogonality, which concept also exists). He then introduces the operation of rotation and distinguishes spinors from tensors by the reversal of sign experienced by the former for a complete rotation. In this space, the vectors, termed kets, and their duals, termed bras, are the most elementary spinors, and Dirac confines his attention to them, since more complicated spinors can be constructed from them by multi-

plications, as done by van der Waerden in his lectures on spinors some decades ago. Their possible application to physical problems is not discussed in detail here, although Dirac does comment briefly about this at several points in the lectures, identifying, for example, the operators for the creation and destruction of fermions.

Dirac then approaches the case of special interest for him, the case of a Hilbert space with an infinite number of dimensions, by taking the limit $N \rightarrow \infty$. For this, the operators of interest are those whose matrix is bounded, so that the operations of multiplication and division are defined for them. This limit leads Dirac to a new class of operators, which do not commute with each other nor obey the associative law of multiplication. He terms these as ‘boson variables’ and they come in two types, identified by Dirac as creation and destruction operators. In the closing section, Dirac emphasises that these boson variables appear automatically in infinite-dimensional theories which start with only fermion variables, provided that the latter are infinite in number. Dirac observes, “There must be such boson operators connected with electrons. Their physical presence is a subject for further investigation. They have a negative energy...”

This book is very much for the specialist, and one may expect it to have much value and influence in suggesting new extensions from the physical theories which we now know. We must note, however, that this little book costs 15 cents per page, which, even given the circumstances, does seem unnecessarily high.

R. H. Dalitz

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The Principles of Human Biochemical Genetics

Second Revised and Enlarged Edition

by **HARRY HARRIS**, Galton Laboratory, University College, London.

Frontiers of Biology, Vol. 19*

1975. 488 pages. Clothbound Price: US \$ 49.95/Dfl. 120.00. ISBN 0-7204-7119-2
Paperback Price: US \$ 20.50/Dfl. 49.00. ISBN 0-7204-4504-3

"...intended not only for those specifically studying human genetics but also for biochemists, biologists and medical men. It is exactly that circle of readers which will find not only information but also inspiration in this book."

Nature

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"...this type of book is a windfall."

BioScience

The above reviews indicate some of the enthusiasm with which the first edition of this book was received in 1970. It went on to become an adopted text by many graduate departments in the United States. The new edition, which encompasses the changes occurring in this large field during the past five years, is undoubtedly one of the most important second editions published on human biochemical genetics. Professor Harris has gathered together and collated extensive information in the field which previously was scattered in numerous journals; the result is a well coordinated text presenting most aspects connected with this area of research. The volume presents a general account of the field while stressing the principle concepts underlying modern research. The net outcome is a clear and well documented account of how inherited diseases and other abnormalities due to inborn biochemical diversity can now be understood as an inherent aspect of the biochemical make-up of human beings.

CONTENTS: Gene mutations and single amino-acid substitutions. One gene - one polypeptide chain. Duplications, deletions, unequal crossovers, chain elongations and other rearrangements. Gene mutations affecting rates of protein synthesis. Quantitative and qualitative variation of enzymes. The inborn errors of metabolism. The blood group substances. Enzyme and protein diversity in human populations. Gene mutations and inherited disease. Appendix 1. Disorders due to specific enzyme deficiencies (inborn errors of metabolism). Appendix 2. Enzyme and protein polymorphisms. References. Subject index.

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Group theory and electronic states

Symmetry of Many-Electron Systems. (Physical Chemistry: A Series of Monographs, vol. 34.) By I. G. Kaplan. Translated by J. Gerratt. Pp. xii+370. (Academic: New York and London, February 1975.) \$34.50; £16.55.

GROUP theory has been used for many years in the quantum mechanical analysis of many particle systems. This monograph deals only with applications to many electron systems, specifically atoms and molecules, the physical properties of which reflect both the symmetry with respect to the permutation of the identical particles of which they are composed and the symmetry of the potential field formed by the nuclei within which these particles move. Other fields of application are ignored.

The book opens with an account of some of the main facts of group representation theory presented as a tool for the chemist, which omits many of the proofs and derivations. The permutation group is singled out for detailed study, an understanding of Young tableaux and Young operators being essential in later chapters. Linear groups of transformations (including the three-dimensional rotation group), point symmetry groups,

and the construction and uses of tensor representations and irreducible tensor operators are all described briefly.

The rest of the book is devoted mainly to the classification and calculation of electronic states of atoms and molecules. Considerable use is made of fractional parentage expansions, which are worked out in detail for the atomic case in $j-j$ coupling and then generalised to handle the non-vector-coupled states that are of importance in molecules. They allow one to construct matrices of one and two-electron operators for an arbitrary molecular system, and in particular to deduce the form of the Hamiltonian matrix. The book ends with an account, specially prepared for the English edition, of Hartree-Fock theory in an orthonormal orbital basis, a survey of methods of treating electron correlation and derivations of self consistent field equations for configurations of spin-degenerate states and for a configuration of non-orthogonal orbitals in the method of 'different orbitals for different spins'.

The calculation of a molecular structure involves a number of successive stages: mathematical methods are first of all needed to formulate appropriate equations for the problem; a numerical method must be devised to solve the equations as economically as possible; the solution must be performed to give

numbers; finally the numbers must be interpreted to give insight into the physicochemical problem under study if possible. The laborious and expensive nature of these calculations reflects the fact that none of these stages are trivial; however, Kaplan only considers the first of them. The group theoretical techniques that he describes are immensely powerful, but are sometimes handled in an uncritical way, as in the use of Young tableaux in the description of the Heitler-London calculation for H_2 . Several types of calculation are described, some of which have not yet been implemented in practice, without any discussion of their advantages or disadvantages, consideration of alternative methods, or presentation of numerical results. This lack of critical discussion means that the book is likely to be of most use to the experienced theoretician who wishes to understand how to exploit the techniques in his own work. The novice would find it tough going and would also be put off by the prohibitively high price.

The translation has been carefully done and I only detected a small number of relatively trivial errors. The original Russian text dates from 1969; consequently, the majority of the references are to literature from earlier years though a handful are of more recent origin.

I. P. Grant

Integrated biology

The Peripheral Arterial Chemoreceptors. (Proceedings of an International Workshop.) Edited by M. J. Purves. Pp. xiii+492. (Cambridge University Press: London, June 1975.) £14.00; \$39.50.

THE carotid body seems to have been the subject of more papers (per weight of tissue) than any other vertebrate sense organ; yet it is probably the least understood in terms of its functional morphology. In recent years this situation has been compounded by the publication of conflicting data, which was the main reason for convening a work session at Bristol in 1973. The present book is the outcome of this meeting.

Unfortunately, the editor gives no indication in the Preface or elsewhere in the book as to what the major unresolved problems and areas of contention are. Furthermore, there is no epilogue to indicate whether, or to what degree, the symposium was successful in resolving any of the controversial areas of research. The reader is therefore left with no alternative but to read the entire book to ascertain where the problem areas lie. Not that this is an unrewarding enterprise: all the articles are well written and of a very high standard.

Perhaps the greatest value in this book lies in the discussions at the end of each

paper, for it is among these pages that the reader is made aware not only of the specific problems in carotid-body research, but also of the subjective nature of the so-called exactness of the scientific method. For example, it is apparent that recognition of nerve processes in electron micrographs is achieved at least as much by intuitive as by objective criteria (page 39).

The subject matter ranges from ultrastructural studies, through neuropharmacological, neurophysiological and metabolic aspects of the carotid body sense organ, to circulatory and respiratory chemoreflexes in the whole animal. In general, the presentation is excellent, which is probably why the few minor editorial faults in the book are so irritating. The electron micrographs are displaced from both text and the legends so that the reader has to keep track of three pages simultaneously if he is effectively to follow the anatomical contributions. Some of the electro-neurograms are poorly reproduced and there are a number of mistakes in the reference lists.

Nevertheless, these faults do not detract from the overall excellence of this book, which can be recommended unreservedly not only to mammalian physiologists, but to all who are interested in an integrated approach to problems in biology. M. P. Osborne and P. J. Butler

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Plasma scattering

Plasma Scattering of Electromagnetic Radiation. John Sheffield. Pp. xii+305. (Academic: New York and London, January 1975.) \$29.00; £13.90.

IN this book John Sheffield covers those aspects of the Thomson scattering of laser radiation by a plasma that are relevant to diagnostic applications. The technique, perhaps the most powerful and elegant to have emerged from work on controlled fusion and other laboratory plasmas, presents particular experimental difficulties in view of the very low ratio of scattered to incident light ($<10^{-12}$ – 10^{-14}). In appropriate circumstances, however, it is unique in the direct relationship between observed quantities and basic parameters such as electron velocity distributions and number densities, and in other cases it provides more complex information. Consequently, various aspects have already formed the subject of many reviews.

Problems arise in devoting an entire book to this topic because of the difficult

choice that must be made between presenting a detailed re-exposition of plasma kinetic theory or assuming a background knowledge and adopting an approach more typical, perhaps, of a review article. By and large John Sheffield copes well with this balance. Though highly specialised, the book will clearly be of considerable use to anyone involved in Thomson scattering measurements, as much for physical explanation and theoretical development as for a reference source. Within the confines of the subject the scope is quite wide, with, for example, chapters covering experimental constraints and optical techniques as equally as, say, the theory of scattering from unstable plasmas. The biggest limitation is that the particular theoretical approach chosen is perhaps not the most suited for comparison with scattering processes operating in systems other than plasmas, so that the book is necessarily limited to a very specialised field within plasma physics. Within that constraint, however, I think the book will prove worthwhile and useful. **D.D. Burgess**

Animal development

Practical Studies of Animal Development. By F. S. Billett and A. E. Wild. Pp. xi+251+6 plates. (Chapman and Hall: London, 1975.) £4.80.

Cellular Interactions in Animal Development. By Elizabeth M. Deuchar. Pp. x+298. (Chapman and Hall: London, 1975.) £6.50.

THERE are several good laboratory manuals available for classes in developmental biology. None is, or could be, comprehensive in the sense of dealing with the provenance and manipulation of all the major developmental systems. Each is of most value to teachers not themselves greatly experienced with the material described. *Practical Studies of Animal Development* by Billett and Wild should encourage enterprising teachers of this kind to attempt new laboratory experiments with their classes. The book provides guidance for work with an unusually wide range of animals—echinoids, ascidians, gastropods, annelids, nematodes, insects, teleosts, amphibians, birds and mammals. The exercises are set in the context of their scientific significance, so the book is not just a collection of recipes. Their selection could not suit everyone, but few teachers of developmental biology will fail to find useful matter in this book. In a second edition it might be worth adding a separate section on organ and tissue culture techniques, giving guidance on the nature and source of some of the materials mentioned (for example, MS 222).

We have had for some time in Ebert and Sussex's *Interacting Systems in Development* an admirable introduction to processes which must necessarily play a big part in any epigenetic event. Deuchar, with her *Cellular Interactions in Animal Development*, now offers a more extended, and more detailed, treatment both of the best known and of many less celebrated cases of cell-interactions in animals. There is virtue in reminding students that the more famous examples form but the tip of an iceberg, and I believe that this book will be successful in exciting student interest not just in ideas, but in the systems on which they can be tested. Not many developmentalists, could hope to survey so wide a range of phenomena as Deuchar does, and although she is still only introducing the reader to the problems she deals with, the end result is a very substantial coverage of those parts of developmental biology that are not 'molecular' or 'genetic'.

Her stories are open ended and she indicates areas of doubt with scrupulous care. I could, indeed, have wished that she had given a more forceful statement of her own views in some cases. It wouldn't matter if some later turned out to be wrong. **D. R. Newth**

Allergic diseases

Immunological Aspects of Allergy and Allergic Diseases. Edited by E. Rajka and S. Korossy. Volume 1, Pp. x+456. Volume 2, Pp. xii+457-759. (Plenum: London and New York; Akademiai Kiado: Budapest, 1974.) \$42 each.

THE unprecedented rate of expansion in immunology during the late sixties and the seventies has not been conducive to the compilation of textbooks. Their place has been taken by numerous excellent monographs of an advanced nature while medical students have been catered for by a number of introductory booklets to modern immunology, mostly of exceptionally high standard. The revolutionary new concepts, however, particularly in this field of cellular immunology, have now become consolidated and these two volumes represent one of the first attempts to contain the avalanche of recent information under one roof. The authors are an almost exclusively Hungarian team and have largely succeeded in producing a reasonably up-to-date handbook of immunology for clinicians, with the special interest of the editors-in-chief in atopic allergy largely underplayed.

The first volume deals with the more theoretical basic aspects of modern thought on immune induction and unresponsiveness, whereas the second discusses diagnostic procedures, transplantation and tumour immunology, with two further volumes of a more clinical nature yet to come. Each chapter is generously supplied with references up to and including 1973.

The occasionally quaint phraseology may charm or irritate, but the text is usually clear and controversial matter well argued. In spite of a chapter devoted to terminological aspects, however, confusion sometimes arises from old and new terms being used interchangeably by some authors, and a much needed glossary is lacking.

On page 349 now hardly relevant theories on reagin structure are given a reference to a much later factual paper containing first numerical data on reagin persistence in skin and showing that reagins do not belong to any then known Ig class, but can be assayed by passive sensitisation of chopped lung, data later ignored in their proper context. There is a somewhat irrelevant though well-written chapter on non-Ig serum proteins. In a detailed chapter on the Shwartzman phenomenon, conclusions about its non-immunological nature are reiterated, ignoring recent evidence on massive complement activation as the trigger mechanism. Immunological aspects of nematode infections are not covered in spite of their recent basic interest as regards reagin formation. And although autoimmunity is discussed at length, modern ideas on the bypassing of antigen-specific T-cell cooperation as a fundamental and for the first time integrated mechanism are ignored. The subject index is deplorably incomplete and there is no author index.

Publication of this book coincides with that of an excellent third edition of an established English textbook covering similar ground at roughly half the price for twice the number of pages. **R. Augustin**

obituary

Lancelot Hogben, FRS, populariser of science and author of *Mathematics for the Million*, died on August 22 at Wrexham Hospital in North Wales. He was four months short of his 80th birthday.

Hogben was born in Southsea of aggressively devout Calvinistic parents, educated in the public schools of Portsmouth and was one of the first batch of County Scholarship boys to enter Trinity College, Cambridge, where he studied zoology. The condescension with which he says he was met left deep and ineradicable mental scars. There, he met and was deeply impressed by the philosopher Bertrand Russell. They remained friends until Russell's death. The influence of Russell—in addition to playing a determining part in Hogben's decision to go to gaol as a conscientious objector in the First World War, although possessed of exemption papers—provides the thread of continuity that ran through the astonishingly wide range of subjects to which Hogben made major contributions. He took nothing on trust, and he explored the fundamentals of each discipline before he expounded it. It was this insistence on knowing what he called the "logical credentials" of each subject that made him—like J. B. S. Haldane—one of the very great "popularisers" of science in the tradition of Clerk Maxwell and Michael Faraday.

After graduation from Cambridge he was lecturer in zoology at Imperial College, London (1919–22); assistant director of the Animal Breeding Research Station, Edinburgh (1923); lecturer in experimental physiology, Edinburgh (1923–25), assistant professor of zoology, McGill (1925–27); professor of zoology, Capetown (1927–30); professor of social biology, London

School of Economics (1930–37); Regius Professor of Natural History, Aberdeen (1937–41); Mason Professor of Zoology, Birmingham (1941–47; during much of this time he was seconded as Colonel to the Directorate of Biological Research, later called Directorate of Medical Statistics, at the War Office, to work with his onetime mentor, and friend, the late Professor F. A. E. Crew); Professor of Medical Statistics, Birmingham (1947–61); Honorary Fellow in Linguistics, Birmingham (1961–63); Vice-Chancellor and Principal, University of Guyana (1963–65).

Besides numerous articles in scientific and medical journals (many of fundamental importance) he wrote a wide range of books ranging from textbooks on zoology through the immensely influential *Primers for the Age of Plenty*, of which *Mathematics for the Million* is perhaps the best known, books on language and linguistics, including *Interglossa* (a blueprint for an international language) and *Astroglossa* (for interplanetary communication) to a hilarious and educative tale, all in words of one syllable, called *Whales for the Welsh*. His two Conway Memorial Lectures, "The Retreat from Reason" and "Authoritarianism and Science" deserve re-reading in the present scientific climate. Hogben was a true polymath. He was honoured with the Keith Prize and Gold Medal of the Royal Society of Edinburgh, a Croonian Lectureship.

That is just the bare bones of what Hogben had achieved. What of Hogben the man, funster, punster, controversialist, socialist, enemy, teacher and friend? That Hogben could be awkward, irascible, impossible and sometimes unreasonable seems, from the frequency with which it has been recorded, to be incontrovertible. He did, however, keep these aspects of his

character in the main for those whom he considered were, in virtue of their position, of equal or superior status. He castigated unmercifully what he regarded as meretricious or intellectually dishonest, but he had patience with the obtuse if he thought they showed a genuine desire to comprehend what he had to impart. He was not very good at what he delighted to stigmatise as "oral intercourse", preferring the written word. Possibly this was because his own speech was "lacunary"—terse and pithy but with whole paragraphs elided—and it took some time to become proficient in interpreting this.

He had an enormously stimulating influence on young scientists, both in his own Department and in others. Birmingham University certainly owed much to him. He would also defend the student interest against illiberal oppression by the dinosauric hierarchy. One instance of this happened at a meeting of the Senate at the University of Birmingham towards the end of the war period. There was a move afoot to cut down the autonomy of the Students' Union, ostensibly because a used contraceptive had been in a fire bucket after a dance. One professor, who for years had almost ruled the Senate, said that, speaking as a father, he was horrified to think that he had sent his daughter to a University where such things were used. Hogben replied that, speaking as a father and a grandfather, he would have been horrified if he thought that he had not. Report has it that after a moment's stunned silence the Senate suddenly dissolved into laughter and the whole episode was seen for what it really was. The next day the President of the Students' Union came to thank Lancelot for what he had done, and the long tyranny of the fossilised one was for ever broken.

announcements

Award

Maurice Strong, executive director of the United Nations Environment Programme, is to receive the **Audubon Medal** from the **National Audubon Society** for his role in making the environment an international concern.

Appointments

Professor Michael Sela is to be the next president of the Weizmann Institute of Science.

The University of Adelaide has appointed **Professor Colin J. Driscoll** to the Waite Chair of Agronomy in the Waite Agricultural Research Institute.

Miscellaneous

The second edition of *Collected Tentative Rules and Recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature* is now available for distribution. Copies can be obtained from:—The American Society of Biological Chemists, Inc., 9650 Rockville Pike, Bethesda, Maryland 20014.

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nature

October 2, 1975

Dear Sir...

Thank you for your application to the Astronomical Research Council for a grant to support the project "Determination of past sunspot numbers from mediaeval records of the swede harvest". The council unfortunately felt that this work was predominantly within the domain of the Vegetable Research Council to whom you are recommended to apply.

Thank you for your application to the Vegetable Research Council for a grant to support the project "A study of the past swede harvests and extraterrestrial influences on them". Unfortunately, the council felt that this work was too interdisciplinary to come within its purview; it recommends you to try the Interdisciplinary Research Committee.

Thank you for your application to the Interdisciplinary Research Committee for a grant to support the project "Stellar magnetohydrodynamics, solar disturbances, atmospheric impacts and their meteorological consequences for agricultural productivity based on mediaeval records". Although the committee felt the research was timely, it feared it did not have enough promise in it. But have you tried one of the foundations—they can often support this sort of work?

Thank you for your application to the Doogood Foundation for a grant to support the project "Man's well-being improved by a better understanding of the influences on agricultural productivity". The foundation felt that the work, although showing great promise, could not yet be regarded as timely. Maybe there are some funds available in your own university.

Thank you for your application to the Miss Hygienia Philpott Fund, for support for your project "A search through mediaeval records within the university". It is true that Miss Philpott's Will did encourage research within the university archives but she added the rider "insofar as this may tend to increase the virtue and godliness of the scholars of the university". The trustees of her Will regrettably could not see such an outcome in this case; surely a government department would support such work?

Thank you for your application to the Ministry of Commerce for support for your project "Improving the balance of payments by a more profound knowledge of the forward market in swedes". We have several laboratories employing thousands of scientists; the project would be ideal to give them something to do, but we couldn't give support for an outsider to do it. Couldn't industry help?

Thank you for your application to the Giant Swede Corporation for support for your project "Better swedes

within the next eleven years". Unfortunately the people in our research laboratory are not too keen on ideas that they have not initiated themselves. Furthermore I should add that the amount of money the swede industry has available for research is very small. Might I recommend you try an organisation with more funds?

Thank you for your application to the Ministry of Defence to support the project "Historical variability in the swede harvest and problems of feeding armies, possible causes". Although it is true that we have substantial funds available for research, we are under growing pressure to demonstrate that this research is relevant to modern military methods. Might I suggest you reword your application appropriately and send it to the Ministry of Attack?

Thank you for your application to the Ministry of Attack to support a project "More effective thermonuclear weapons through better understanding of time-dependent thermonuclear processes in the Sun". Unfortunately the Minister is increasingly worried by the damage that students are causing to university laboratories at which military research is being done. Maybe you could disguise your work with a humanitarian purpose.

Thank you for your application to the Cancer Foundation to support a project "Do sunspots cause cancer directly or through swede harvest variability?" I'm afraid we found the proposal a little far-fetched. Maybe a private benefactor would help.

Thank you for your letter to the Twelfth Marquess of Cumberland requesting support for your project "Harvests were better in the olden times". Unfortunately His Grace is a trifle penurious these days what with swingeing taxation, and he writes from Bermuda to recommend that you follow his example of collecting money in small quantities from large numbers of people in return for some favour that you can bestow upon them.

It has been brought to the attention of the Director of Public Prosecutions that you together with some students were to be seen last Saturday dressed as swedes and offering the public a look through a telescope at the Sun in return for 10p to "support research". Since I do not find your name on the list of registered charities licensed to collect in the streets, I must warn you that any repetition of this offence will lead to prosecution. Besides, there are perfectly normal channels for obtaining money for research purposes; perhaps you are unaware of the government's handbook which lists them (alphabetically) as the Astronomical Research Council, . . .

... Your obedient servant



IN his first major statement on energy policy, President Ford last January declared that he firmly supports a massive expansion of the nuclear energy programme in the United States. Without such an expansion, he argued, there is little hope that the nation can free itself from its painful dependence on foreign sources of oil, and he announced that his Administration would strive to ensure that at least 200 nuclear power plants are generating electricity by 1985. That would represent almost a fourfold expansion, from the present 55 nuclear stations.

With such a strong verbal commitment from the President, backed by more than \$1,000 million in the present federal budget for a variety of nuclear energy programmes, it might be supposed that the nuclear industry is in the midst of a bonanza. But it isn't. Instead, it is under siege, and it is facing a very uncertain future.

It is caught up in a complex web of technical, regulatory and economic problems, under assault by articulate and resourceful critics, facing increasing scrutiny by the Congress, and unsure of its public support. In fact, the problems are so serious that earlier this year a government task force concluded that "the nation's nuclear power program is approaching a state of crisis", and it added that it "is not optimistic that in the restrictive environment in which the nuclear industry operates today . . . actions can be implemented quickly and completely enough to avoid significant restriction on the growth of fission energy use". The task force which made those statements consisted of some of the top officials in charge of the federal government's nuclear energy programme.

One of the most acute problems afflicting the nuclear industry is an economic one—the national economic recession has more than made its mark on the industry. New orders for reactors, which not very long ago were coming in thick and fast from electric utility companies, have slumped recently, and the utilities have also been forced to postpone or cancel scores of orders which they placed before the recession began to bite. The figures speak for themselves: in the 18 months which ended on June 30, firm orders were placed for only 34 reactors, 17 reactors ordered previously were cancelled, and construction of 167 others was postponed for periods ranging from six months to indefinitely.

On top of those acute—and possibly short term—economic woes, the industry finds itself operating in a new and, its supporters insist, more hostile political environment in Washington. The demise earlier this year of the Atomic Energy Commission (AEC) and its replacement by the Energy

The first fuel bundle at the Duane Arnold Energy Center is lowered into its slot in the nuclear reactor, February, 1974.



Nader, anti-nuclear leader.

Energy policy in the United States assumes that there will be a marked expansion of the nuclear power programme, with as many as 1,000 nuclear power stations in operation by the year 2000. But the nuclear industry is now experiencing some of the toughest political and economic challenges it has ever faced. In the first of three articles on U.S. nuclear policy, Colin Norman reports from Washington.

Research and Development Administration (ERDA) demolished the bureaucratic structure which nurtured the early growth of the nuclear power programme, leaving the industry devoid of a solid power base in the federal government to look after its interests. Nuclear energy is now just one of five divisions in ERDA, and it must compete directly with other technologies for its share of the energy research and development pie.

And the industry's political base in the Congress is also fast being eroded. Congress is just beginning to take a searching look at the nuclear power programme, and for the first time in nearly 30 years, the powerful Joint Committee on Atomic Energy, which has jealously guarded the interests of the nuclear programme, has been forced to yield some of its monopoly over atomic energy affairs. A subcommittee of the House Interior Committee, under the chairmanship of Representative Morris Udall—a declared candidate for the Presidency—has been conducting prolonged and well publicised hearings on some of the more controversial aspects of the nuclear power programme, and the Congressional Joint Economic Committee has also been delving into the tricky questions of nuclear energy finances. Moreover, the Joint Committee on Atomic Energy is itself an entirely different animal from what it was just a year ago; two of its longest serving, and most powerful, members have left Congress, and the committee has benefited from an influx of more sceptical members of Congress.

This altered political environment sprung up just as a number of controversial issues, which will fundamentally affect the future growth of the nuclear industry, are coming to a head at the national level. In the next few years, the federal government will have to decide the structure of the uranium enrichment industry, how to dispose of radioactive waste material, whether to allow plutonium produced by reactors to be recycled as reactor fuel, and whether a new generation of breeder reactors should be built.

But, although those issues affect the long term structure of the industry, a more direct challenge to present-day nuclear operations is beginning to emerge at the state and local level. For the past four or five years, applications to build nuclear power plants have been challenged by local environmentalist and anti-nuclear groups, intent chiefly on keeping nuclear power plants out of their neighbourhoods. The groups have operated, more or less in isolation, by fighting the nuclear industry on a case-by-case basis. But recently their activities have become more coordinated, and a loose coalition has formed with

with a charismatic and highly effective leader—Ralph Nader.

The political effect of the local anti-nuclear groups is already being felt. According to a survey carried out in May this year for the Atomic Industrial Forum (AIF), the nuclear industry's trade association, there are bills pending in virtually every state legislature in the country which would to some extent curb the growth of nuclear power at the local level. Though very few of the more restrictive measures stand any chance of being passed, the sheer volume of the legislation is causing some concern among nuclear advocates. But a new development is causing even more concern.

Earlier this year, a coalition of environmentalist groups in California gathered together more than the 313,000 signatures needed to call a state-wide referendum on nuclear power next year. The referendum, in the form of a proposition which will be placed on the ballot papers for the Presidential primary elections in June, is a cleverly worded resolution which would forbid nuclear plants from being built in California unless they meet stringent safety criteria, unless safe waste-disposal measures have been devised, and unless the public is fully insured against the consequences of nuclear accidents. Moreover, if existing power plants in California don't meet the standards (which at present they don't), the proposition states that they would have to be gradually phased out of operation over a period of several years.

Ed Koupal, a Californian who organised the petition, is quick to point out that the measure "is not against nuclear power, it is for nuclear safety", but the net effect of the proposition—if it is passed—would clearly be to impose a moratorium on nuclear power in California. Though its chances of being passed are generally considered to be slim, there are two strong factors working in its favour. First, the concept embodied in the proposition—that nuclear power should only be allowed if it can be shown to be safe—is easy to sell in an election campaign. And second, a new California state law drastically limits the length of a campaign and the amount of money that can be spent in a state-wide election, which means that the nuclear industry cannot launch a massive propaganda blitz to get the measure defeated.

California isn't the only state likely to hold a referendum on nuclear energy matters next year. A group of environmentalist organisations, loosely coordinated into a coalition called the Western Block, is trying to get resolutions similar to the California proposition placed on the ballot papers in the November Presidential elections in 16

other states.

In short, therefore, the nuclear energy industry finds itself facing some of the toughest political and economic problems it has encountered in its troubled history, while, at the same time, the conventional wisdom among energy planners in the federal government is that a massive expansion of the nuclear programme will be vital to meet growing energy demand.

The industry is gearing up to put its case across to Congress and the general public. The AIF, for example, is stepping up its public relations campaign; it has moved its headquarters from New York to Washington, doubled its budget to \$1.2 million a year, and is already sending out reams of paperwork to the news media. AIF officials say that they do not plan to do any direct lobbying because another industrial organisation has been set up for that purpose. Called the Nuclear Energy Council, it is headed by former Representative Craig Hosmer, who was the ranking Republican on the Joint Committee on Atomic Energy until he retired from Congress last year.

In several respects, the industry has a number of advantages over its critics in its bid for national attention. Not only is the AIF able to outspend the nuclear critics by almost an order of magnitude, but it is much better organised and has far greater resources to draw on. Moreover, it recently received a considerable boost from a public opinion poll, carried out by Louis Harris. The poll revealed that 69% of the public would approve an expansion of the nuclear power programme in the United States, while only 19% of the respondents declared themselves opposed to such a move.

The chief nuclear critics operating at the national level are Ralph Nader's organisation, the Union of Concerned Scientists (UCS) and the Natural Resources Defense Council (NRDC). The UCS has provided most of the technical backing for challenges to the safety of nuclear power plants, and its principal leaders are Henry Kendall, an MIT nuclear physicist, and Dan Ford, an economics graduate. The NRDC has been most active in attacking health standards associated with plutonium, safeguards against the theft of a weapons-grade nuclear material, and the government's arguments in support of the liquid metal fast breeder reactor programme (LMFBR). Tom Cochran, a nuclear physicist, Arthur Tamplin, a former AEC health physicist, and Gus Speth, a lawyer, form the basis of NRDC's nuclear attack force.

The debate on the desirability of nuclear power has, of course, been going on for several years, but its focus has shifted considerably. Early con-

cern about the health effects of the releases of small amounts of radioactivity from power plants, and worries about the environmental effects of thermal emissions, have greatly diminished—chiefly because the standards have been considerably tightened. The most controversial issues now are the safety of light water reactors, hazards associated with plutonium, the proliferation of nuclear weapons, and the difficulty of finding a safe method for disposing of highly radioactive reactor wastes. Those issues will ultimately have to be resolved by Congress.

Congress will have to make up its collective mind on the following matters.

Energy demand. An underlying feature of the entire nuclear energy debate is a fundamental divergence of opinion on energy policy. The nuclear critics, in short, argue that if stringent, mandatory energy conservation measures are applied, and if research and development on technologies for using solar energy are stepped up, the nuclear programme can be slowed down and there will be no need to develop the breeder reactor. The most forceful backing for that viewpoint was incorporated into the findings last year of the Ford Foundation's Energy Policy Project, a massive analysis of energy options which cost \$3 million to complete. But the Congress has so far shown no stomach for taking the unpalatable political decisions needed to slow down energy growth, and federal energy policy is firmly based on the assumption that, although energy growth may be slowed a little, the growth in demand for electrical energy will continue to be large. In the past few years, consumption of electrical energy has been increasing by about 6% a year. Consequently, in order to stave off increased oil imports, new generating capacity will have to be provided by coal and nuclear fission.

Those assumptions are firmly embodied in a long term energy research and development plan published on June 30 by ERDA, which will form the blueprint for ERDA's operations over the next few years.

Reactor safety. In the past few years, the central issue in the nuclear power debate has been whether or not the many safety features built into light water reactors can reliably guard against a major accident which would release large quantities of radioactivity into the environment. The focus of the debate has been an arcane piece of plumbing known as the Emergency Core Cooling System (ECCS), which is designed to flood the reactor core with water if a pipe breaks and the reactor loses its main supply of coolant.

The issue of the reliability of the

ECCS was first brought to public attention by Kendall and Ford of the UCS, who successfully badgered the Atomic Energy Commission into holding prolonged hearings on the matter in 1972 and 1973. Though the hearings failed to resolve the dispute, the AEC tightened its operating standards for ECCS in December 1973, and it is planning a full scale test of the device in an experimental reactor in Idaho in the late 1970s. The critics have not been slow in pointing out that scores of reactors are already being built with an essentially untested safety device.

Some of the steam has, however, been taken out of the safety debate by a massive analysis of the probability of a catastrophic reactor accident, which was carried out for the Atomic Energy Commission by Professor Norman Rasmussen of MIT. A draft of the report, published last year, offered the widely quoted conclusion that the probability of a severe accident resulting in the release of large quantities of radioactivity, is extremely small, that it would result in about 2,300 deaths, and that it would cause about \$6,000 million of damage to property. The report has stimulated a good deal of controversy, however, and some of its findings have been challenged by a separate analysis by the American Physical Society.

In the meantime, however, a fire which occurred in March at the Browns Ferry nuclear power station in Alabama—the world's largest nuclear power plant—reopened the safety dispute. Caused by an electrician checking for air leaks with a candle, the fire knocked out the ECCS system at the plant, and raised a number of questions about the ability of plant operators to react to an emergency.

Plutonium and the LMFBR. Most of the present controversy surrounding the nuclear power programme is concerned with the possibility that plutonium, produced in nuclear reactors, may be recycled and mixed with uranium as reactor fuel. Worries about the toxicity of plutonium have arisen, chiefly in connection with a theory put forward by Cochran and Tamplin of the NRDC, that tiny inhaled particle of plutonium could lodge in the lungs, providing the surrounding tissues with a massive dose of radioactivity. Though the theory has been hotly challenged by various scientists, it is still giving rise to considerable debate. But a more prominent and worrying problem with plutonium is the possibility that quantities of it could be stolen from the nuclear industry and fashioned into crude nuclear bombs, either by terrorist groups or by countries which possess nuclear reactors, but which have not yet produced nuclear weapons. India's development of a nuclear explosive

from plutonium derived from a reactor supplied by Canada has brought the problem to public attention rather dramatically.

Because of uncertainties surrounding the adequacy of safeguards to prevent illicit diversion of plutonium from the nuclear industry, the Nuclear Regulatory Commission (NRC), which has assumed the regulatory functions of the AEC, announced earlier this year that it will probably defer making a decision on whether or not to allow plutonium to be used as a reactor fuel until 1978.

The nuclear industry is upset by the NRC's delay because, it argues, plutonium recycling will be needed to supplement dwindling supplies of uranium; it would reduce requirements for enriched uranium by 10% in the early 1980s. Congress may well have to decide the matter eventually, since a bill outlawing plutonium recycling until the uncertainties have been resolved has been introduced into both the House and the Senate.

Plutonium recycling is itself, however, only a prelude to introduction of the breeder reactor, which is designed to produce more plutonium than it consumes as fuel. The breeder reactor programme is the single most costly energy research and development project supported by the federal government, but its advantage is that, whereas light water reactors would run out of uranium fuel in a few decades, the breeder could provide power from fission for several centuries.

Major disagreements have erupted over the timing of the programme, its cost—the research and development effort alone is expected to swallow up about \$10,700 million, compared with an estimate just three years ago of \$3,300 million—and whether or not other, more acceptable, technologies can be developed in time to make the breeder redundant. A major challenge was offered to the budget for the breeder programme in Congress this year, but it was defeated.

Waste disposal. A major uncertainty afflicting the nuclear programme at present is what will eventually be done with the highly radioactive waste material produced in the reactor core. Although it is generally expected that burying the wastes deep in a salt mine will eventually prove to be safe and acceptable, the concept has yet to be thoroughly tested, and a suitable site has yet to be found. A site was prepared for testing in Kansas in the late 1960s, but it was found to be punctured by abandoned oil and gas wells; the project ran into spirited local political opposition and it had to be scrapped. Consequently, the Atomic Energy Commission announced that it would store radioactive wastes above

FUNDS to support research are becoming scarcer, so it is increasingly important to ensure that the money available is used as profitably as possible. For some years I have been suggesting that we are getting poorer and poorer value for the public money voted for research, and that an increasing fraction is being wasted on unnecessary and complicated administration. One of the fears aroused by the Rothschild recommendations was that they would make for additional complications and further waste. Even the most enthusiastic supporters of these changes cannot deny that there are many more scientists, individuals who were previously themselves engaged in research at the bench, who are now desk-bound planners, and that research funds are also spent on many more non-scientific administrators supporting (or even controlling) the scientific administrators. The hope is that this will result in the remaining bench workers devoting their time more profitably to more important projects.

In the meantime, it may be useful to try to find what sort of organisation is producing the most for the least expenditure. In the past, I have been accused of sentimentality for looking back nostalgically at the small units which operated efficiently in the 1930s, when funds were even tighter than today. I have been told that it is impossible to "put the clock back". The assumption is that the age of string, sealing wax and enthusiasm has gone for ever.

I have therefore been looking with interest at the performance of the Oil Pollution Research Unit of the Field Studies Council. Here we have a small group of young scientists working in converted cellars and outbuildings on a smaller total budget than that enjoyed by many individual academics who have received research council grants for studies which have made little noticeable impact on science or human welfare. At an international conference held at Aviemore in Scotland in April of this year, and sponsored by the Institute of Petroleum and the Field Studies Council, members of the unit described their work. It was clear that this was both important and entirely relevant to the needs of the petroleum industry and to understanding the likely ecological effects of the exploitation of oil reserves. The work fulfilled all the

Lab charges



KENNETH MELLANBY

requirements that the Rothschild proposals are intended to produce.

The Oil Pollution Research Unit (OPRU) has, however, shown that a very high proportion—more than 90% I would estimate—of their funds are actually spent directly on research, paying salaries, equipping laboratories, hiring facilities and visiting sites. The parent body, the Field Studies Council, is itself an admirable organisation, with the least top-heavy administration of any comparable body. They do not have time to interfere in the running of the OPRU, though they are available to give help where this is required. The main lesson to be learned from this research organisation is, once more, that "small is beautiful", or at least that the small unit can still be the least wasteful.

I think that many other organisations could learn from this example. Their overheads are very much higher, though their productivity is, on the whole, lower. We hear many complaints from government departments, who have funds to commission research, that some laboratories run by research councils, or even by other government departments, are in danger of pricing themselves out of the market. This is because most contracts must bear a surcharge of 100% to meet overheads and administrative costs. It is difficult not to suggest that these could be greatly reduced by a simpler administrative structure.

There is even some criticism of university departments, which are

accepting contracts and making a much smaller surcharge. This is, in some circles, considered to be unfair competition. It is suggested that they should be compelled to charge more, for they are accused of not costing their work properly. As one with some experience of university administration, I do not think that this is the case, though the argument might appeal to empire-building registrars wishing to increase their own staff numbers. If I, as head of a department, had accepted a research contract, and it had included all the costs of staff and their equipment and materials, I would not have spent a penny more on running the department, nor would there have been any need for the central university administration to increase its costs. I might have had to write an occasional letter, but I would have hoped to compensate for this, and more, by persuading the research workers to give a few of my lectures on subjects in which they possessed a particular expertise. I would have looked upon my relationship with the contract team as one of symbiosis, and not treated them as parasites.

My main criticism of many university and polytechnic staff today is that they often assume that they cannot do worthwhile research unless they can land a substantial grant for that purpose. Even junior lecturers think they are justified in asking for a graduate research assistant. Yet even when they obtain this extra money, many are obviously less productive than the pre-war lecturer who was lucky if he could obtain a few pounds from departmental funds for extra materials. It may well be that the present shortage of money will have its good results. Research workers may have to eschew problems requiring costly apparatus (much of which is accumulating dust up and down the country) and devote more time to thinking how to tackle problems within their own resources. They may even realise that some of the large grants which have been floating about in recent years have actually been counter-productive. We are said to be facing, in Britain, a financial crisis even more serious than that experienced in the 1930s. We should therefore remember that, in many subjects, it was then that Britain led the world in scientific research. □

the ground in a temporary facility until an ultimate waste disposal method has been found. Earlier this year, however, ERDA announced that it was reconsidering the whole problem and would issue a statement on the matter in due course.

With those disputes unresolved, and

reactor sales at a virtual standstill, the immediate prospects for President Ford's goal of bringing 200 nuclear reactors on line by 1985 looks slim. But, in the absence of a strong, coherent energy policy featuring mandatory energy conservation and massive support for non-nuclear energy

technologies, the nuclear power programme represents the only proven alternative to greatly increased oil imports. Faced with that prospect, Congress will be forced soon to take some controversial decisions. In the meantime, the nuclear energy industry will have to live with uncertainty. □

international news

At the opening last week of the annual conference of the International Atomic Energy Agency in Vienna, the Director General, Mr Sigvard Eklund, expressed confidence in the continuing expansion of nuclear power technology. He suggested that nuclear energy based on fission provided the only immediately available alternative to fossil fuels in spite of recent optimism about the potential of alternative energy sources.

Addressing the delegates of 107 member nations of the IAEA Mr Eklund said that as alternative resources from solar, geothermal and wind power, and from nuclear fusion would not become readily available for some time and that even then those sources would provide less power than current estimates suggested.

Mr Eklund was, however, optimistic that largely under the guidance of the IAEA the problems associated with the use of fission reactors could be overcome. These included three main issues which have recently formed the focus of public debate, namely, the safety and reliability of the reactors, the disposal of radioactive waste, and the need to ensure that the spread of nuclear technology does not lead to a proliferation of nuclear arms development among the non-nuclear powers.

Dealing with the first of these points Mr Eklund said that the IAEA is engaged on a programme to formulate a comprehensive system of international

IAEA head puts faith in nuclear power

by Allan Piper

safety codes and guidelines for nuclear power plants. The agency believes, he said, that the "teething troubles" undergone by the larger reactors have now been eliminated, and that it is no longer fair to regard nuclear power stations as unreliable.

Many of the problems associated with the satisfactory management of waste products remain to be resolved, however. In particular, the IAEA is establishing a standing advisory group to examine the feasibility of burying waste in geologically stable terrain. Referring to that concept Mr Eklund drew attention to the point raised during an earlier agency conference that plutonium formed in the Oklo Formation about 1,700 million years ago decayed quite harmlessly without escaping into the environment.

On the question of preventing an escalation of nuclear weapons proliferation as more countries obtain the materials for developing nuclear power plant Mr Eklund stressed the need for the adoption of internationally accept-

able IAEA safeguards. He expressed disappointment that though the Non-Proliferation Treaty Conference held in Geneva in May expressed strong support for safeguards controlled by IAEA their recommendations had not been considerably more decisive.

Calling upon the present nuclear powers as the major exporters of nuclear materials and technology, Mr Eklund urged them to take the lead by supplying nuclear resources only to those nations prepared to place their "entire nuclear activity" under international safeguards.

He stressed that commercial and political interests should not prevail over that objective, adding that further progress towards a comprehensive ban on nuclear tests by the nuclear powers themselves would assist by setting an example to the developing countries. He also proposed that the explosion of nuclear devices necessary to the development of nuclear technology be placed under international control.

Though Mr Eklund's speech reflects a confidence in the future of nuclear fission as a major source of energy he indicated that IAEA are not discounting altogether other possible sources. The agency, together with the World Health Organisation, is, he said, co-operating with the International Institute of Applied Systems Analysis on a programme to evaluate the viability of every available option. □

M. d'ORNANO, the French Minister for Industry, has presented the conclusions of the consultative commission on energy, chaired by M. B. Gregory, the Director General of the CNRS (National Centre for Scientific Research). This commission was set up to examine the future direction of government energy policy, and includes representatives from a very wide spectrum of interests—specialists, energy users, and "social partners"—so that all shades of opinion can be expressed. Three criteria were drawn up to help the working of the commission—low financial cost, political independence, and ecological and social aspects, but the commission "regretted that it could not report within this framework any coherence in the proposed courses of action". In fact, the commission's report is not always in agreement with decisions reached by the government.

In its plans for 1985, the commission

places special emphasis on efforts to reduce energy consumption. By comparison with 1973 forecasts, the total consumption hoped for would be 240 Mtpc (millions of tons petroleum equivalent) instead of 285 Mtpc, and the amount derived from petroleum products would be 96 Mtpc instead of 178 Mtpc. The report stresses that "this is compatible with an annual growth rate of 5.5% only at the price of a policy involving strict restraint, but it is well worth the effort to attempt to reach this goal". The feasibility of the 5.5% rate as the basis of a working hypothesis has, in any case, been thrown into doubt by some members of the commission. Coal consumption (30 Mtpc) would continue to decline progressively, while gas consumption would rapidly increase to 37 Mtpc.

The recommendations of the commission regarding electricity are very

precise: whereas electricity production would, at the present rate, have approximately doubled by 1985, the commission underlines the dangers of a policy exclusively favouring electricity, it urges that major research and development efforts be made on solar, hydrogen and other energy sources.

The most striking point of the report is without doubt the split which has appeared within the commission over the nuclear programme. Some of its members advocate, for safety reasons, the construction of nuclear power stations of lower output than that proposed by the government, the balance of the energy being provided by traditional power stations even if this seems less favourably economical. M. d'Ornano has perhaps shown excessive optimism in making the report of the Gregory commission public, for it seems to call into question many aspects of government policy. □

France boosts nuclear physics

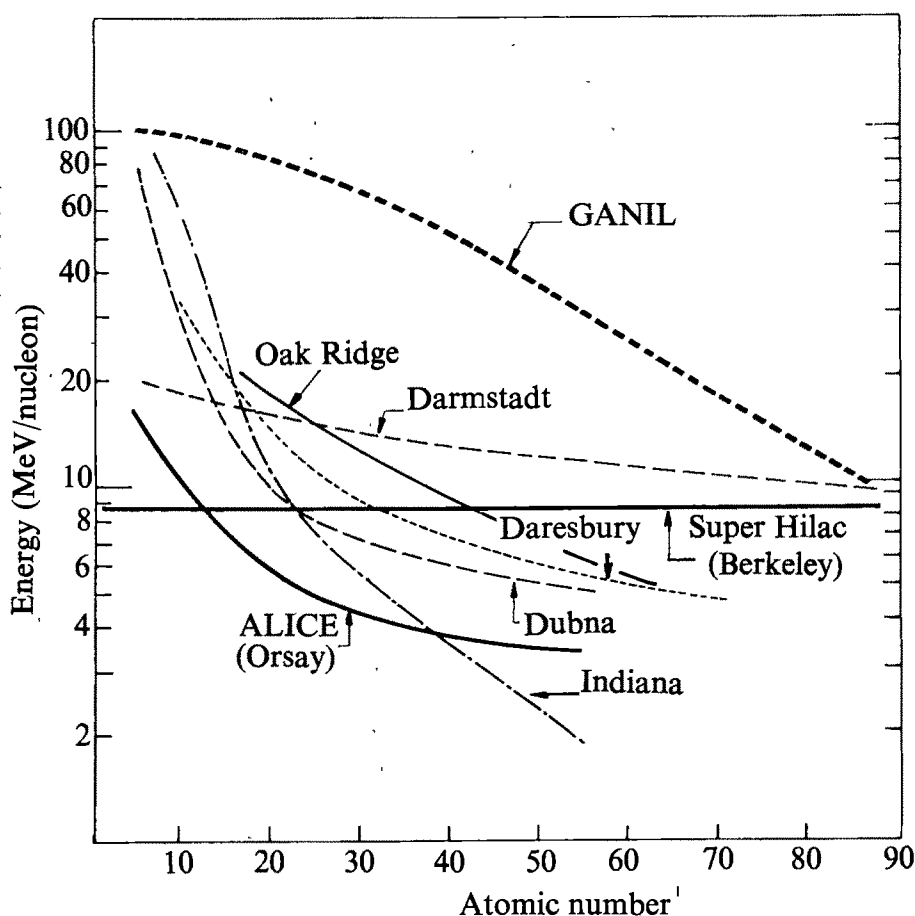
by Roger Woodham

A NEW experimental facility for 'low energy' nuclear physicists, to be known as the Grand Accélérateur National à Ions Lourds (GANIL), will be included in the French Seventh Plan which covers the years 1976-80. It will cost about £20 million, should be in operation before 1980 and is to be built just outside Caen in Normandy.

As well as being part of the French government's strategy for livening up the French economy by authorising spending on new scientific projects during the next five years, the Caen decision also has implications for the recently acquired French interest in regional policy. With the mood in France at present much in favour of decentralisation, the Délégation Générale à la Recherche Scientifique et Technique (DGRST) has taken the first tentative step towards some sort of regional policy in science by giving the green light to GANIL.

Instead of choosing to build the new accelerator complex in the vicinity of Paris, perhaps at Saclay or Orsay, the DGRST and the Minister of Industry and Research, M. Michel d'Ornano, settled on Caen—a somewhat surprising selection which was not on the list of places like Grenoble, Strasbourg, Lyon and Bordeaux which have been widely canvassed during the past year or so. Cynics like to point out that M d'Ornano is Mayor of Deauville (not far from Caen) and was until last year President of the Regional Council of Lower Normandy. The fact is, however, that Caen is only two hours by a fast train service from Paris and will therefore be easily accessible to nuclear physicists from other countries, a significant consideration given that the French see GANIL as an important international facility.

The GANIL project, which will make available beams of heavy ions ranging from carbon to uranium at energies higher than is or will be possible with machines elsewhere, is the brainchild of a coordinating committee set up by the DGRST in 1972 to look at the whole matter of French support for nuclear and elementary particle physics. (In France these activities come under the auspices of the Commissariat à l'Energie Atomique (CEA) and the Institut National de Physique Nucléaire et de Physique des Particules, the latter being an umbrella organisation colloquially known as IN2P3 and covering all nuclear and particle physics activity in university laboratories and those of the CNRS (Centre National de la Recherche Scientifique).) GANIL was



A comparison of the energy per nucleon available from GANIL (planned) compared with that available (or planned) at other accelerators

dreamt up by a working party drawn from the CEA and IN2P3, and the money required will be provided in equal measure by the two organisations.

There are two somewhat unusual features of GANIL: first, that the main part of the system is to comprise two large cyclotrons, one of which will inject particles into the other, and second that superconducting magnets, which are increasingly in evidence in designs for new accelerators, are not to be used. As to the cyclotrons, the GANIL team maintains that it is only by using this kind of accelerator that high values of the energy per nucleon of a nucleus can be achieved. Certainly, if the promise implied in the figure is upheld, the French will have something to be proud of: over much of the range of atomic numbers the energy per nucleon available from GANIL is several times higher than from other accelerators.

The GANIL team decided against superconducting magnets mainly on the grounds that France does not have enough experience to take what might amount to a very risky step, especially as others have hit snags while building such magnets for accelerators. The team argues, however, that a cyclotron system is absolutely necessary and is

to be preferred to the tandem Van de Graaff arrangements to be used at Daresbury in the UK, for example. Certainly the linear machines at Darmstadt and Berkeley, for example, are capable of accelerating greater numbers of particles per second, and the quality of the beam from a linear machine is on the whole better than from a cyclotron, but when it comes to increasing the energy delivered, the cyclotron seems to win hands down.

Not all users of GANIL will have to avail themselves of the maximum energy available, however. Many of the people who will eventually use the facility are chemists, biologists and so on, and it will often be that they will use beams of particles from the first cyclotron, before they are injected into the second one for further acceleration. In any event, the site for GANIL—which is still a green field on which no more than preliminary geological investigations have been made—is close to the Faculty of Medicine of the University of Caen, and to a college of technology; there is also ample room for interested industrial concerns like electronics companies to set up their own bases nearby so that they can make use of GANIL for their own investigations, on ion implantation, for example. □

ONE of the more practical ideas to come out of the Stockholm Conference on the Human Environment in 1972 was that of setting up some sort of central service for the collection and dissemination of information on a very wide range of environmental matters. Before the conference, some people seem to have envisaged a centralised service actually providing information, or at least providing direct references to precisely where it could be obtained. But a more realistic look at the situation, and in particular an appreciation of the wide range of subjects and problems to be covered, indicated that this would not be feasible, at any rate on a global scale. Even if a centralised system had been a practical proposition, it would be quite impossible to keep information up to date, especially with environmental affairs in their present unstable and rapidly developing state.

In fact, what the conference organisers had had in mind all along was an obvious alternative, in the shape of a referral service. This would not itself provide or store information, but would aim to put its clients in touch with those places where the information they seek could be found, and to do this in an orderly, internationally accepted fashion. After two years of careful planning and consultations with governments, research institutes and other organisations with relevant experience, the International Referral Service (IRS) has recently started to operate. In view of the wide field to be covered, the UN Environmental Program (UNEP) decided to base the service on a number of priorities considered by members of its Governing Council to be of global importance. Because the service must also be useful within individual countries, however, or to clients having purely local interests, it needs to encompass a wider definition of the general concept of "environment" than is generally understood by the UNEP itself in its day-to-day working.

A service that does not itself provide information, but is designed to indicate where that information can be found, will obviously have smaller storage requirements than a centralised direct information service, and will not need such extensive updating facilities; nonetheless, more is needed than the UNEP can provide, although it is not in fact necessary even for the basic information to be centralised. As now set up, therefore, the IRS works through a number of 'focal points', notionally one for each contributing country. A typical focal point has two roles: first, it acts as a centre for the collection of information and, second, it functions as a subsidiary of the IRS within its own country. It will thus be able to

operate in two ways:

- By collecting and storing detailed information not required by the IRS but needed locally.
- As part of the overall IRS network. Operation at the purely local level recognises the fact that most countries now either have, or intend to have in the near future, some sort of environmental control mechanism, which will, however, inevitably trespass on areas already covered by other government departments. The sets of information available within countries are in fact largely in the areas so covered, and

UN environmental service launched

from Peter Collins, Geneva

the national environment organisations are thus best suited to cataloguing sources of such information within their own countries.

Being financed by government and therefore liable to government control, a national focal point also controls itself as regards its clientele. It is expected to evaluate its own sources and to keep out trivial requests, thus giving the system a good deal of flexibility and national control. The only clients other than those approved in this way by the focal points are the members of the UN system, who could be among the biggest users of the new service, and non-governmental organisations having official status. At the other end of the chain, the national focal point can also decide whether or not to accept a request, although it is not anticipated that this sanction will often be applied: a request already passed by the focal point in its country of origin is unlikely to be rejected. The answer to a request goes back through the same channels as those through which it was received.

From the practical point of view, the essential document is the IRS Operations Manual, the international version of which appeared in August this year. This comprises clear and concise guides to the various elements of the service—sources, focal points, and users, giving the purpose and responsibilities of the first two and the procedures to be followed by the last. Because the service must be operable on a multilingual basis, and is essentially designed for computer handling, a complete list of codes is given, any given attribute having the same code number in any language. These codes cover sponsorship, activities, functions, geographical coverage, working language, availability, output and subjects. Two further sections provide computer logic and computer search guides. Initially designed for use with

the programming languages COBOL and PL1, in both of which programs are already available, the system can be adapted to any modern computer, UNEP believes, in less than a week, and plans for conversions to other languages are already in hand.

In practice, a question is coded and passed to the computer, which selects those sources, details of whose stored or accessible information most closely fit the coded question. The answer describes the source (for example, government-sponsored laboratory, research organisation, university department), gives details of its coverage and lists those subjects most (and less) relevant to the query. The client then selects the source or sources that seem most likely to have the information he requires, and contacts them. The UNEP reckon that the cost, as far as the IRS is concerned, is about \$2 an answer. This has, of course, no connection with any fee payable by the user to the selected source.

Some 10 focal points are already operating the IRS, and another 40 or so should begin functioning within the next few months, including a regional one for the CMEA countries and, apparently, the USSR (although Hungary and Yugoslavia will have their own). The manual is available in English, French, Russian and Spanish. Alone of the official UN languages, Chinese cannot be used as it is not suitable for use with a computer. There are already translations into Danish, Dutch, German, Greek, and Italian. The UNEP has held the first of a series of training programmes in the use of the service in Nairobi. One will follow shortly in Geneva, on the use of relevant computer techniques, then two in Dakar (in French) and Mexico City (in Spanish).

The basic idea behind the IRS is to cover the environment in the broadest sense, recognising that people know their own disciplines but need information on others. Countries holding environmental information are not, they point out, necessarily the most advanced technologically; Kenya, for example, has much more experience of running National Parks than have many European countries. The aim at present is to run the service on the basis of the present manual for two years, then to appraise the situation to see what revision is needed. But as it stands, the IRS provides a good example of how a UN agency can function in the way most people think it should: as an international liaison office and clearing house, passing information between member countries or institutions within those countries, and so organised that everyone using the system knows what to expect and can be reasonably sure of getting it. □

As the much delayed jubilee celebrations of the Soviet Academy of Science finally approach, it is perhaps inevitable that the role of the scientist in Soviet society should become a matter for editorial comment in the Soviet press. The relationship between pure research and the immediate needs of the state plans is always a delicate one, and a saving clause referring to the ultimate benefit to the national economy is regularly included in the description of fund-hungry projects of the space programme. The closer connection between the pure scientist and the needs of industry has been one of the fundamental themes of the present five-year plan; this aspect of Soviet science is being stressed in the press preparations for the jubilee. The concept is one fundamental to Soviet thinking—Lenin himself envisaged a total reconstruction of the Soviet economy in which the Academy would play the major role in providing the Soviet Union with “the possibility of equipping itself independently with all the principal forms of raw materials and industry”. Seen in this light, the comment of a *Pravda* editorial (September 20) seems merely an exhortation to renewed efforts: “The duty of a scientist as a citizen is to appear as a champion of scientific and technical progress not only at the writing-desk or blackboard but also to fight for the cause outside the walls of his study or laboratory as well”. The reference recalls, however, the press campaign of September 1973 against Academician Andrei Sakharov, who was accused of betraying his chief duty as a Soviet scientist—that of being a patriot. Sakharov’s “offence” was, of course, his liberal political views, not an excessive preoccupation with pure science. Nevertheless the *Pravda* editorial does suggest that, once again, the slogan of patriotic duty is being used to urge scientists into a greater conformity with Party policy—in this case, the devoting of their talents to the immediate needs of the economy, even at the expense of potentially more valuable basic research—a somewhat ironic way, surely, of celebrating the 250th anniversary of the Academy of Sciences.

● Following recent speculation on the latest Soviet explorations in the Weddell Sea a number of statements made in the Soviet media and through the *Novosti* agency have set out the programme for the latest (twenty-first) Soviet Antarctic expedition, which is due to set out in October. This will include atmospheric sounding, the investigation of deep-level ice cores already taken by advance parties, oceanological investigation in the

Drake Straits, participation in the international South Pole Experiment (Polex South) and a massive survey of the mountainous hinterland of the Weddell Sea. The expedition, led by candidate of Geographical Sciences G. I. Bardin, is to be a large scale one, involving 255 scientists, a flotilla of five ships, including the flagship, the new research vessel Mikhail Somov, an Ilyushin-14 aircraft, four helicopters and five heavy tractors. It will also convey to the Antarctic a considerable amount of new equipment for existing Soviet bases.

Soviet diary

from Vera Rich



● During the past few years, a number of Franco-Soviet joint research projects have taken place, notably in the fields of geophysics and space research. Less publicised has been a biochemical experiment—perhaps because the research was carried out by an all-Soviet team from the Institute of Biochemistry of the Soviet Academy of Sciences, with France providing only some of the specimens. The purpose of the investigation was to establish the chemical constituents of the “bouquet” of champagne. The method used was that of gas-liquid chromatography; the test specimens were of both French and Georgian vintages. It was found that the bouquet is attributable to certain high-boiling-point constituents of fusel oils, notably terpenoid compounds of *cis*- and *trans*-farnesol, and also certain complex esters—ethyl linoleate, ethyl lactate, ethyl caproate, phenylethyl acetate and so on. This contradicts the previous assumption that the bouquet is attributable to highly volatile constituents. Small quantities of highly volatile compounds are, in fact, involved, but these form only a general ‘background’.

● The development of a silk industry independent of China was one of the great aims of the Byzantine emperors, who, when they at last obtained the

secret of silk-worm rearing, promptly made the industry an Imperial monopoly. From emperors to State Planning Committees seem a far cry, yet the Soviet Committee for Science and Technology has recently declared the establishment of native a silk industry to be one of its major inter-industry problems.

The new Soviet silk production is not to be based on the mulberry-feeding Chinese silkworm, but on the caterpillar of the oak egg moth, which is traditionally cultivated in Korea and northern provinces of India. After some 20 years’ research and selection, the Ukrainian Agricultural Academy has succeeded in producing a strain of insect which can be cultivated successfully in the harsher conditions of Poless’e and other areas of Ukraine. By means of hybridisation and selection, an insect has been produced with a life cycle compressed into a single season. Professor N. N. Sinitskii, who was responsible for the project, claims that now silk production need no longer be confined to the Central Asian Republics of the USSR, but can flourish “from the Carpathians to the Urals—wherever there are oakgroves”.

The effect on the ecology of Ukraine and the other western republics of the USSR as not been mentioned. However, in the Irkutsk provinces of western Siberia, another major research project has just been completed; a long term study of the best biological method for combatting a major pest of the Siberian forests—the Siberian silkworm.

● Thirty years ago, largely as a result of the havoc wrought by the Second World War, the European bison was a vanishing species. Now, as a result of careful conservation work in the Byelovezhchaya Forest (which is managed jointly by Poland and the Byelorussian SSR), it has made a remarkable recovery. Partly as a result of this capacity for survival, a team of zoologists in Vologda are attempting to produce a cross between the bison and domestic cattle. Several examples of the new hybrid have already been produced, and they have bison-like features, including increased body-weight, bluish-black hair, a slight hump, and straight sharp horns. The hybrid calves show considerable hardiness, being able to drink ice-cold water immediately after birth and having a considerably more rapid rate of growth during the first 10 days. The hybrid has proved to be fertile, and the first calving from the hybrid cows is due this autumn—after which the fat content of the milk can be established and compared with ordinary cow’s milk. It is hoped that the hybrid will preserve the high yield of the domestic cow.

correspondence

First-year ecology

SIR,—The teaching of ecology at university first-year level probably poses more problems than any other area of biology. Although in general there is agreement as to its desirability, opinions differ considerably as to the methods of implementation. Wide-spread public interest in topics such as conservation and pollution, reflected in pressure from students for courses in ecology, provide powerful reasons for attempting to overcome curriculum problems. We also believe that in an introductory biology course ecology should be presented not only as a subject in its own right but also as having important links with other fields of biology such as physiology, behaviour and genetics. Furthermore, it must be interdisciplinary, involving zoological and botanical aspects, as well as incorporating discussions of the role of micro-organisms in different ecosystems.

To meet these requirements at Bath, we have been reviewing the teaching of ecology at the first-year level in which there is provision for ten one-hour lectures and five two-hour periods of practical work. Our lecture/seminar programme centres around three principal themes—spatial distribution of organisms, the flow of energy in ecosystems and the gene as an element of continuity in populations. On the practical side we have accepted the fact that, at the most, only one field trip will be feasible and we exclude the possibility of vacation courses at this level. The main problem is the practical work which must necessarily be restricted almost entirely to the laboratory and be carried out in the minimum of time. It is also important that, being the first year, various investigations should work. We have therefore directed planning towards the possibility of using simulation experiments with living organisms, many of which have already been tried out in smaller and more advanced classes. The value of these experiments lies in the fact that they aim to reproduce and illustrate in miniature within the laboratory certain selected aspects of the real environment. Study involves not only qualitative description but also quantitative sampling and statistical treatment of results, together with simulation experiments covering such topics as succession, population growth, selection and the estimation of animal

numbers by means of mark, release and recapture experiments.

Simulation, however, has serious drawbacks since it demands extrapolation by the student from imitation to reality. Such an intellectual jump can pose problems. Although in the teaching of ecology, there is no substitute for the experience of a visit to a natural habitat, the introduction into the lecture component of visual aids, such as ciné films, still photographs and slides can help to bridge the gaps.

The inception of a new curriculum should also be associated with a fresh range of objectives some of which will bear on the cognitive field and some on the affective. It is not therefore enough merely to change the nature and quantity of the course content; the methods of teaching and learning must also be adjusted to suit the new requirements. This is particularly true in a complex subject such as ecology which tends to face students initially with a bewildering diversity of problems. Such needs have served to underline the changing role of the lecturer and tutor who acts not only in his traditional capacity of expositor, but also as guide, consultant and stimulator of discussion.

W. H. DOWDESWELL

I. C. POTTER

University of Bath

Cheating children

SIR,—I would like to comment on two letters in your issue of September 4 which are related to my work on metal bending. The first is that by Dr Pamplin and Mr Collins, whose interesting study of deception in spoon-bending leads me to ask what is the proportion of children who cheat in other forms of activity in which they are given the opportunity to deceive. I have also seen cases of obvious fraud in my initial tests and have not continued working with the subjects in such cases. I have found so far a ratio of no more than one in six of those who claim metal-bending powers (at least forty subjects) to have been able to achieve the effect in a manner which I cannot explain by deception. This is consistent with the results of Pamplin and Collins.

I would like to suggest that the criteria of bending might have been made more stringent before children were tested in this way, and even while the one-way mirror tests were being performed. Apparently some of the

children still claimed they could spoon-bend without cheating after they had been caught. They might still be able to do so, though only if the conditions are stringent enough will it be possible to get them to turn their attention to using any abnormal powers they possess. The measuring of pressure or at least having a strip of metal taped flat to a desk so it cannot be mechanically bent is necessary here.

In response to Mr Randi, who was very proud of his ability to be able to cheat all and sundry, may I say that his last sentence has remarkable hubris. I would be grateful to learn of Mr Randi's scientific qualifications before I can accept it, and I echo the apt words of the great scientist Sir William Crookes, a past President of the Royal Society, by insisting that when it is necessary to trust my senses I maintain that a physical enquirer is more than a match for a professional conjurer.

May I also recommend to Mr Randi the report of his fellow magicians from Atlanta, A. Zorka and A. Dickson who tested Mr Geller under conditions they felt excluded fraud.

Yours faithfully,

JOHN TAYLOR

King's College, London, UK

Assays

SIR,—With reference to the article of A. Dorozynski (September 11), I would like to make the following comments:

1 The mutagenicity assay, elaborated by Dr Bruce Ames, has been used by the IARC in Lyon for three years within the framework of a continuous collaboration with Dr Ames. Dr Bartsch of the Unit of Chemical Carcinogenesis of the IARC has devised some modifications of the original procedure which have allowed an improvement of the testing efficiency.

2 The IARC is collaborating, and is certainly willing to continue and expand its collaboration, with a number of national laboratories, including the Institut Pasteur in Paris, on the development of screening tests to detect chemical mutagens and/or carcinogens.

3 The IARC is not, and will not be, involved in the commercial dissemination of the "mutatest"

Yours faithfully,

L. TOMATIS

*International Agency for Research on Cancer,
Lyon, France*

news and views

IN the world of professional archaeology of the 1920s and early 30s, the fact that you were a 'Glozelian' or 'Anti-Glozelian' determined who were your friends; this was particularly significant in an academic discipline not unknown for acrimonious controversies. The reason for this in-fighting was based on a reported discovery of artefacts which to most archaeologists were—and to many still are—unacceptable and were therefore labelled as fakes. The first pieces were reported in 1924 by a local farmer, Emile Fradin, who is still very much alive living in the same farmhouse at Glozel, an isolated village south of Vichy in France; these discoveries were quickly followed by many more undertaken by a M. Morlet, a doctor from Vichy. What caused the furore was the fact that these objects were like nothing seen before anywhere in the world and covered an amazing spectrum of different types. There were clay tablets with mysterious incised inscriptions in an unknown language, jars and other ceramics with and without inscriptions, pottery phallic symbols, animals inscribed on bone and pebbles. None of these objects was recognisable archaeologically and even if accepted as something new, many found it impossible to reconcile the different periods represented apparently cheek by jowl; for instance what might conceivably be Neolithic incised stones were apparently found in the same context as clay tablets with inscriptions, which, some said, represented early Iberian scripts.

The controversy raged more or less continuously until the War in 1939. During this time two international commissions of inquiry sat and pronounced exactly opposite decisions and some five law suits were fought with varying results. In Britain the balance against the Glozelians was heavy although on the continent such eminent archaeologists as Professor Reinach were strongly opposed to the notion that deliberate forgery had taken place.

There the matter rested until 1974 when Glyn Daniel, Professor of Archaeology at Cambridge University, decided to dot the i's and cross the t's in his lecture on archaeological fakes and forgeries; he would have a few pottery samples from Glozel tested using the thermoluminescent (TL) technique in order to illustrate their modern date. Exactly the opposite result transpired—the TL measurements clearly indicated

The Glozel affair

from E. T. Hall

that the ceramics were not modern, but seemed to have been manufactured in the Gallo-Roman period (*Antiquity*, December, 1974).

These results, which were later more thoroughly confirmed by further measurements, have again divided the archaeologists. To some the antiquity of the ceramics in particular is no great surprise, although the admixture of these ceramics in the same context as other less acceptable material, such as engraved bone and pebbles of apparently Neolithic date (which are less amenable to dating techniques), makes the unravelling of the whole story very difficult. To other archaeologists the affair is still a distasteful hoax and TL dating must be in error; for them it is similar to telling a physicist that the laws of thermodynamics are no longer valid and, not surprisingly, they find it difficult even to listen.

Where then can the explanation lie? At the symposium on Archaeometry at Oxford University in March, details of the work undertaken by McKerrell at Edinburgh and Mejdahl in Denmark were given, and discussed at length by both physicists and archaeologists. There can now be little doubt that the ceramic material is not modern; preliminary dating results on the bone using the ^{14}C technique tend to support the TL measurements. It has been suggested by some that the ceramics, which were low-fired, could be ancient material such as tiles which had been reshaped and inscribed recently. Though this is a possibility with some tablets, with others it is ruled out because on close examination small bubbles of vitrification can be seen in the grooves of some of the inscriptions. Others have suggested recent deliberate irradiation by X rays or γ rays to give the correct TL dates; such possibilities are ruled out by comparatively simple tests which have indeed been carried out. However it is generally agreed that the archaeology associated with the digging is most unsatisfactory. No properly controlled digging took place except for attempts by both commissions of inquiry of a very limited kind and when 'planting' of finds seemed a distinct

possibility. There is, therefore, no scientific evidence that all of the objects were in fact from the same stratigraphical context or even from Glozel itself.

It is important in these discussions to differentiate between authentication and dating by TL measurements. When a ceramic object has been removed from its immediate surroundings and has been kept in a museum for some years the extent of the radioactive bombardment from the surrounding soil (as opposed to the internal contribution) becomes a matter of conjecture. Dating by TL measurements of such an object must then have much wider limits than when the precise environment is known and an accuracy of $\pm 10\%$ or better can be expected; when the burial environment is unknown the dating accuracy must be subject to greater errors, although in the instance under discussion the approximate level of soil radioactivity would be known provided, of course, that the objects were in fact excavated from the Glozel area. In the case of authentication however we are dealing with a different order of magnitude; in this case we are deciding between an age of 50 and at least 500 years: an error of 1,000% or more. The Glozel measurements would seem to show without doubt that the ceramics are not modern fakes, but it might be a little rash to differentiate with any complete confidence between, say, a Gallo-Roman and a mediaeval provenance. The idea of a mediaeval date becomes a clear possibility after the work of Huxtable and Aitken reported in the latest issue of *Antiquity* (September 1975), albeit on a single specimen. Such a date is perhaps more likely since an archaeologically acceptable mediaeval kiln was discovered on the site. They also point out that McKerrell and Mejdahl's results are based on only five dates and it will be interesting to see, when full publication is made, whether all the objects sampled fall into a coherently dated group, taking into account the wide range inherent in this type of TL measurement.

How does TL dating come out of the controversy? There have during the past four years been some dramatic exposés of modern fakes which had been unsuspected until the advent of TL dating; many Tang wares from Hong Kong, Hacilar from Turkey, Amlash from Tehran and massive Etruscan terracottas from Italy have

all been shown to have been manufactured during the past few years on a large scale and are (or were) decorating shelves in private collections or public museums around the world. These results are now accepted almost unanimously by the relevant authorities and there have been no obvious instances where results undertaken by a reputable laboratory have been shown to be in error as far as authentication is concerned; in other words, so far TL authentication has been shown over many hundreds of samples to be reliable. It would seem unlikely that the Glozel ceramics should be an extraordinary exception to this story of reliability particularly since they do not exhibit any anomalous behaviour during measurement and give entirely normal TL glow curves.

A number of the archaeological objections to the authenticity of the site would be removed if it were found that certain of the objects

were genuine and some false. For instance; one might postulate that the higher fired tablets were genuine, but the weird face urns, phallic symbols, bone and pebble carvings were not; if the ceramics in the latter category had been fabricated from tiles or bricks fired in antiquity and reconstituted, this could give an explanation of their apparent ancient TL date. Measurements being undertaken at Oxford using archaeomagnetic techniques may give some clues as to whether this is a possibility.

It is to be hoped that the projected scientific re-excavation of the site by French archaeologists will proceed apace—a preliminary magnetometer survey has already been undertaken. Although the site has been extensively and randomly dug by many different excavators, if the site is genuine, some objects must remain and their archaeological contexts may help to explain this perplexing problem. □

Polymorphs and amorphs

by Robert W. Cahn

THE central puzzle about glasses is this: why do some melts form glasses on slow cooling while others do not? As with all such broad questions, the golden nugget of understanding recedes further and further as hypotheses pile up. If an explanation is attempted in terms of the viscosity/temperature relation, then one wants to know what determines this reaction; if in terms of critical nucleus size and diffusion rates, then again one asks how these are related to chemical composition. As more and more unconventional glass-formers are discovered, it becomes increasingly difficult to establish what chemical or structural features they have in common. A radically novel hypothesis therefore deserves close attention.

Such an hypothesis is advanced in this issue of *Nature* (page 370) by C. H. L. Goodman. The central observation on which his hypothesis is based is: "A surprisingly large number of the systems which form glasses have as a major (sometimes the only) constituent, a material that exists in two or more polymorphic crystalline forms which differ but little in free energy", and he goes on to exemplify silica, BeF_2 , PbO , As_2O_3 , TiO_2 , Se, S and CdP_2 . He then asks whether polymorphism could be a necessary condition for a material to be a glass-former. The rest of his extremely interesting and well-documented paper is devoted to exploring, admittedly in purely qualitative terms, the implications of his novel hypothesis.

He suggests that, above the glass transition temperature T_g , transient

"flicker-clusters" of all possible polymorphs should coexist, as happens for instance in liquid water. The crucial point here is that none of these clusters can reach a viable size for crystal growth, because the various clusters of polymorph A would be impeded by the clusters of polymorph B, and in particular the separate A clusters could never achieve mutually epitaxial orientation relationships. As the melt cools, eventually the clusters become firmly bonded across parts of their surfaces, leaving liquid-like material in other, unbonded interstices. This stage corresponds to T_g . The role of the minor additives that assist glass formation might well be to concentrate in the residual interstices and reduce the strain resulting from differential thermal contraction of the distinct A and B clusters. (Goodman regards it as an important test of any hypothesis whether it can interpret the important role of some glass-modifier additives which have a major glass-stabilising effect in quite small concentrations.)

Goodman suggests various experimental tests of his ideas: for instance, ways are available to check on the coexistence of microcrystalline clusters and liquid-like interstitial layers. Also, recent work on the radial distribution function in vitreous silica, combined with dilatometric and high-pressure studies, have yielded evidence for the simultaneous presence of several micro-polymorphs. He goes on to point out certain categories of materials which on the basis of his hypothesis should not form glasses: there are thus the begin-

nings of a predictive capacity for the model.

Particularly interesting is the final section of Goodman's paper, where he suggests possible practical implications of his ideas. Thus, the solid cluster/liquid layer model implies that near T_g a glass might behave as an effective absorber of gases, like a zeolite, and points out that this is consistent with the known action of glass films on the surface of semiconducting devices. He suggests that carbon melts containing 'solvent catalysts' such as iron or nickel (used for diamond synthesis) might be capable of being quenched into the glassy state. Finally, he suggests that Griffith cracks, which determine the tensile strengths of glasses, might be controlled by the microstress systems and chemical heterogeneity of his postulated heterocluster arrays. He does not say it, but it might well be that the scale of the cracks (and hence strength level) would be determined by the size distribution of the clusters, and one then becomes interested in what determines this distribution!

One feature of many oxide glasses to which Goodman does not address himself is the observation that many (perhaps most) such glasses undergo liquid-liquid separation above T_g : electron microscopy and X-ray small-angle scattering both show that disperse drops of liquid separate out in a matrix of liquid of distinct chemical composition. It is not immediately clear whether Goodman's hypothesis would imply that each liquid phase needs to break down into arrays of two (or more) types of polymorphic microclusters, or whether it suffices if one phase does so. In any case, it would appear that in view of the prevalence of liquid separation, Goodman's ideas imply that there are often two distinct levels of structural heterogeneity in a stable glass.

This paper will suggest to the reader all sorts of new consequential hypotheses and various kinds of experiments to follow them up. Whether or not time fully confirms his ideas, his paper will undoubtedly prove to be of seminal importance. □

Bølling interstadial in Britain?

from Peter D. Moore

THE elucidation of climatic fluctuations at the close of the last (Devensian) glaciation in Britain is fraught with difficulties (see *Nature*, 251, 185; 1974). One of the major controversies centres upon the events leading up to the so-called Allerød interstadial; in continental Europe a warm period (termed the Bølling interstadial) is believed to have preceded the Allerød,

and there is some indication of another warm period before the Bølling, called the Susaca interstadial (van der Hammen and Vogel, *Geol. Mijn.*, **45**, 33; 1966). In Britain, evidence even for the Bølling is scant and it is widely believed that its influence upon contemporary vegetation was of such a nature that it cannot be distinguished from the succeeding Allerød. Not all palaeoecologists are of that opinion however.

Last year Burrows published the results of his analysis of plant macrofossils from a late Devensian lake site at Nant Ffrancon in North Wales (*New Phytol.*, **73**, 1003; 1974). This site had previously been worked upon by Seddon (*Phil. Trans R. Soc.*, **B244**, 459; 1962), who described a typical sequence of sediments and fossils suggestive of a cold/warm/cold development in late Devensian climate. The new core studied by Burrows, however, revealed a rather different sequence. The organic sediments, often indicative of the Allerød interstadial in British sites, was found, and above and below it were clays with a stony content and with macrofossils indicative of colder conditions. But within the lower clays, about 26 cm below the supposed Allerød, were silty muds, rich in organic matter, and containing plant fragments indicative of relatively warm climatic conditions, including tree birch and *Filipendula ulmaria*. Naturally, this stratigraphic profile, with two organic layers, leads one to speculate that the Bølling interstadial has at last been found in Britain.

It seems very strange, however, that the Bølling should make its effects felt in the mountainous regions of Snowdonia, and yet should not be evident in the many lowland sites which have been studied. In support of his contention, Burrows quotes the data of Crabtree at Cors Geallt, just 10 km from Nant Ffrancon. There, a similar but very thin, organic band was reported in the pre-Allerød clays (*VIII^e Congrès INQUA, Paris*, **1**, 217; 1971), but the arrangement of pollen sampling within the core in question does not permit its clear interpretation as the product of a Bølling interstadial.

The interpretation of cores, either in temporal or climatic terms, on the basis of their stratigraphic sequence alone is a risky business. One needs absolute dates, in this case radiocarbon dates, before such a sequence can be understood. Burrows has recently published some dates from his Nant Ffrancon core (*New Phytol.*, **75**, 167; 1975), but unfortunately the difficulties are still not resolved. Of the four dates he has obtained, he is forced to dismiss the lowermost two as gross underestimates, possibly resulting from contamination. The lowest acceptable

date comes from the basal layers of the upper (supposed Allerød) organic layer, and is $11,900 \pm 500$ b.p. The commencement of the Allerød is conventionally dated at 11,950 b.p. (Godwin and Willis, *Proc. R. Soc.*, **B150**, 199; 1959), so Burrows' date confirms these deposits as belonging to the Allerød interstadial, as it is normally understood in the British context. Although this date still allows the Bølling interpretation which Burrows sets upon the lower organic layers, it does nothing to confirm this. When one takes into account the possibility of rapid sedimentation of the intervening clays and the large standard error of the date quoted, it is still possible that the lower muds also belong to the traditional Allerød.

The case for an undisputed British Bølling therefore still remains open. A more important fact emerges from this involved controversy, however. Despite a plethora of regional information on late Devensian sequences within the British Isles, we still have no type section at which our distinctively British sequence can be defined. No amount of pro-European feeling can excuse the casual and largely inaccurate use which we make of such terms as 'Allerød' and 'Bølling', which have only been defined adequately in their continental context. An intensively studied British type site might at least remove some of the suspended sediment from these currently disturbed and cloudy waters. □

Photoperiodic regulation of insect growth

from our Insect Physiology Correspondent

THE arrest of the process of growth in insects that is known as diapause may occur at different phases of embryonic development, during larval growth, in the pupal stage or in adult reproduction. Diapause used to be defined as an 'inborn' arrest because it appeared to be uninfluenced by environmental changes. But that was because the 'sensitive period', during which induction of the arrest takes place, often occurs long before the actual arrest appears, and the inducing factor was therefore overlooked. In recent years increasing attention has been given to the nature and regulation of the sensitive period itself. A recent paper by D. S. Saunders (*J. Ent. (A)* **50**, 107; 1975) deals with these phenomena in the flesh-fly *Sarcophaga argyrostoma*; it is an instructive short paper because it reviews and confirms earlier observations on this insect (and other insects), contributes some important new discoveries, and generally illustrates the modern approach to the subject of diapause.

The flesh-fly *Sarcophaga* has a diapause controlled, as usual, by photoperiod. Sensitivity to photoperiod begins while the embryos are within the maternal uterus (in these flies the eggs hatch within the common oviduct) and continues throughout larval development, to end at the time of puparium formation. A short daylength of less than 14.5 h in 24 h gives a high proportion of diapausing pupae; larvae raised at a long daylength exceeding 14.5 h give rise to pupae with uninterrupted development. As in other insects a definite number of short- or long-day cycles is required to induce the necessary switch. As the author had earlier shown, these two variables (1) the length of the sensitive period, that is, the length of larval life and (2) the number of short-day cycles required to raise the proportion of diapause pupae in one day's batch to 50%, have different temperature coefficients.

The length of larval life is highly dependent on temperature ($Q_{10}=2.7$), whereas the 'required day number', as defined above, is largely compensated for temperature change ($Q_{10}=1.4$ or less). Consequently, at high temperature (26 °C) the larvae reach puparium formation and their sensitive period comes to an end before the required number of short-day cycles has been experienced, and development proceeds without interruption. Whereas, at a low temperature (16–18 °C) the sen-



A hundred years ago

THE *Photographic News*, in speaking of "Photography and the Illustrated Press," gives some examples of the extent to which the latter is now dependent on the photographic art. The *New York Daily Graphic*, besides often executing its pictures from photographs, employs a photo-mechanical process in the production of some of its work. At the office of the *Moniteur Universel*, which is one of the most extensive printing and publishing establishments in France, arrangements are being made for large photo-printing works, as well as for producing coloured pictures by M. Leon Vidal's photo-chromic process. In this country photography is used to aid the artist in sketching to a great extent. One of these days, no doubt, the *News* believes, we shall have our papers illustrated by photographs *pur et simple*, but even now photography has far more to do with the execution of the illustrations in our journals than most people may be aware of.

from *Nature*, **12**, 503, October 7, 1875.

sitive period of development is prolonged, sufficient short-day cycles can occur and the incidence of pupal diapause is high. Moreover, the length of larval life is shorter at long daylengths than at short daylengths and this will reinforce the effects of temperature.

The author now tests this interpretation by exposing larvae to short-day cycles (LD 12:12) and borderline temperatures of 18 and 20 °C, and then varying by other means the duration of larval development and so the sensitive period. The most effective means of accelerating puparium formation, and so curtailing the sensitive period, was by overcrowding larvae in a limited quantity of meat; and as predicted this reduced the incidence of pupal diapause. (Premature extraction of third instar larvae from the culture, or exposure to pure CO₂ for 24 h had comparable effects.) The sensitive period was lengthened by allowing larvae to wander in wet sawdust, which defers puparium formation; this treatment increases the incidence of pupal diapause.

The theory that insect diapause results from an arrest in secretion of the hormones necessary for growth (or reproduction) was put forward more than 40 years ago and is now fairly generally accepted. Precisely how photoperiod controls hormonal activity is not known. In general it is thought that long or uninterrupted nights are inductive in the diapause-promoting sense, whereas short or interrupted nights are inductive in the development-promoting sense. There is evidence that both effects are accumulated by the photoperiodic counter, the former to a threshold at which, for example, brain hormone secretion is suppressed, the latter to a point at which secretion is released, thereby leading to ecdysone secretion by the ring gland, puparium formation, growth, and eclosion. What is it that is being summed? The author argues convincingly that the summation of successive photoperiods cannot be the summation of cryptic effects of ecdysone. It seems more likely that the mechanism for summing photoperiodic cycles lies in the brain itself where the release of the brain hormone is regulated. □

How birds reach home

The 14th International Ethological Conference was held at Parma, on August 27–September 5

from John R. Krebs

THE problem of how migrating birds and homing pigeons navigate still

remains unsolved in spite of intensive research efforts in the past few years. Over twenty years ago, Gustav Kramer suggested that in order to fly from an unfamiliar place to a geographically distant home site, a bird would need information analogous to a map (on which to read its own position and that of home), and a compass (to choose the appropriate direction indicated by the map). It is now well established that both migrating song birds and homing pigeons (on which much of the experimental work is done) have up to three types of compass—based on the Sun's azimuth, star patterns and the resultant of the Earth's magnetic field, but the nature of the map remains elusive.

F. Papi and his colleagues from Pisa presented a synthesis of their experimental evidence supporting the idea that pigeons use an olfactory map of windborne smells to home from an unfamiliar release site. The results are impressive. In one experiment three groups of pigeons were reared in outdoor aviaries side by side. Two of the groups were exposed to abnormal wind directions by an arrangement of screens that deflected the wind through 90° clockwise or counterclockwise, and the third group acted as a control. When the pigeons were released 105 km from home, the control group headed straight home, the group exposed to clockwise wind rotation headed off 65° counterclockwise from home, while the counterclockwise group deviated clockwise by a similar amount. In a second series of experiments, pigeons were raised in outdoor aviaries and exposed to artificial winds from the North or South bearing the scent of olive oil or α -pinene. The birds were later released 26 km West of home, and flew South if a drop of 'North-wind' scent was placed on their nostrils before release, while they flew North if treated with the 'South-wind' scent. These and other experiments all suggest that the birds learn to associate particular scents with winds coming from particular directions towards the home loft, and that they use this information together with the concentration of scent at the release site, to construct an olfactory map showing the position of the release site in relation to home.

Although the Pisa group's results look convincing, W. T. Keeton (Cornell University) reported that he was unable to replicate them, and the reason for this discrepancy is unclear, although as one Italian delegate suggested, the Italian countryside, redolent with the aroma of olives and garlic, may be more conducive to olfactory navigation than are the extensive deciduous forests of upstate New York. In any case, it seems much less likely that an olfactory map would be used by long distance migrants travelling thousands of miles from

northern temperate regions to the tropics. Many of these birds travel at night, and for some years it has been known that they use the pattern of the stars as a compass. W. Wiltschko (Frankfurt University) reported some very neat experiments showing that the star compass is 'set' according to the magnetic compass that these migrants also possess. Wiltschko recorded the spontaneous migratory fluttering movements of garden warblers and robins in small circular cages. When the birds were allowed to see the sky on a clear night in a normal magnetic field, they fluttered in the usual migratory direction, roughly South in the autumn and North in the spring. When magnetic North was artificially rotated by 120°, the birds correspondingly altered their heading, even though the stars were still visible. Robins took longer to adjust than garden warblers, so they seem to take more notice of the stars. Wiltschko then showed that the robins could be trained to use a totally artificial pattern of 'stars' as a compass, if they were first shown the pattern in association with the Earth's magnetic field. The magnetic field is used to 'calibrate' the star pattern, which can then be used on its own. One may well wonder why the birds use a star compass at all if it has to be set with reference to a magnetic compass; perhaps it requires longer to 'read' the magnetic compass, so that the visual compass is used for quick reference while the bird is in flight. Finally, the big remaining mystery surrounding the magnetic compass, is how the bird's sensory system detects the Earth's magnetic field. □

Conservation of marmosets

from J. P. Hearn

An international conference on the Biology and Conservation of Marmosets convened by the National Zoological Park, Smithsonian Institution was held at Front Royal, Virginia on August 18–21.

MARMOSSET monkeys are becoming more common in laboratories and rarer in the wild. The potential of marmosets as models in human oriented studies in immunology, virology, reproduction, contraception, teratology and other fields, is just starting to be recognised, but already some species are impossible to obtain and several South American countries have banned the export of marmosets completely.

Recently, sixty scientists from the United States, South America and Europe participated in an International Conference on the Biology and Conservation of Marmosets and the picture that emerged gave cause for concern.

Several species of marmosets—the lion tamarins, the buff headed marmoset, the white eared marmoset and the cotton topped tamarin (*Leontopithecus* species, *Callithrix flaviceps* *Callithrix aurita* and *Saguinus oedipus oedipus*)—are threatened so severely that they are near extinction. The major threat to these animals is the clearance of their natural forest homelands. The lion tamarins from Brazil are now dependent on only 2% of their original natural range and not more than 600 are thought to survive in the wild.

Three suggestions were made to rectify the situation: A total ban on the export, shooting, or interference with the endangered species; urgent studies in the wild by census and observation; and careful management of the remaining home environment. These animals depend on particular types of trees for food and nesting holes. Planting of such trees, with shelter holes artificially bored in them if necessary, and a supply of additional foods, would assist the survival of these animals in the present unnaturally reduced environments to which they are restricted. As any delay in the application of effective measures may well prove disastrous, the conference asked for assistance from conservation organisations and the governments of the source countries.

Marmosets used in research are usually the common marmoset, the saddle-backed tamarin and the moustache tamarin (*Callithrix jacchus*, *Saguinus fuscicollis* and *Saguinus mystax*). These are not immediately threatened but may become so as growing research needs outstrip the replacement of natural wild stocks. The threats to their continued supply from the wild are the appalling losses suffered at present during shipment from the wild to the user countries and the accelerating rate of deforestation in developing South American countries.

In this case there are several longer term solutions that can be applied. Marmosets, and in particular the common marmoset, are prolific breeders in and out of captivity. They can be maintained in self-replacing colonies that are inexpensive to run compared with colonies of the more conventional, larger laboratory primates. Therefore, breeding centres should be established on a large scale commercial basis both in the countries of origin and the countries where marmosets are used in research. Laboratories should also develop colonies that are self-replacing in order to become independent of supplies from the wild.

In the shorter term it is essential that the conditions in which marmosets are held between capture in the wild and arrival in the laboratory be greatly improved. Of necessity this control must be applied in the source countries, by the rigorous application of export quotas and by inspection at the export centres. At present only about 10% of marmosets caught in the wild survive the various stages necessary before they become established in the laboratory. This has resulted in an unreal escalation in demand. The situation could easily be improved as marmosets are amenable and adapt very easily to captivity if given the right conditions.

At a time when the need for primates in research is growing and supplies from the wild are dwindling, many scientists are looking to the smaller new world monkeys as an alternative to the more conventional macaques. The marmosets are receiving particular attention as they are small, inexpensive, easy to manage and breed well in captivity, but if their exploitation is not more carefully managed than it has been in the last five years there will be disastrous consequences for the wild populations. □

New light on luminescence

from G. F. J. Garlick

An International Conference on Luminescence was held in Tokyo on September 1–5. The full proceedings will be published in an issue of the *Journal of Luminescence* in 1976.

New lamps for old might have been the cry at many previous luminescence conferences, particularly because of the developments in fluorescent lighting, electroluminescence and cathode ray tube phosphors. This year in Tokyo, however, it was a different transition. Having seen its offspring, the laser, safely launched, luminescence research is utilising the high intensity and short pulse lifetime features of lasers to enter a new phase. It is now possible to look at the very early stages of excitation and at the fascinating effects of high excitation densities. It is no longer legitimate to separate the emission processes from absorption and excitation and one can hardly sustain the definition of luminescence which distinguishes it from the Raman effect. The invasion of the pre-relaxed states of both inorganic and organic systems by experiment and theory was well described in the various review papers at the conference. The process of luminescence is now

analogous to the 'compound nucleus model': the luminescing system interacts coherently with the exciting radiation field and relaxes by various channels. K. K. Rebane (Institute of Physics, Academy of Sciences of Estonia, USSR) showed that it is possible to look at the unrelaxed states at ordinary excitation levels in the steady state but there seems to be no doubt that picosecond techniques and high intensities are needed to sift out the detailed stages from coherence interaction to the dephased, relaxed state. As exemplified by the large number of original contributions, high intensity studies reveal, through their characteristic luminescence spectra, new entities such as the interacting exciton-photon—the polariton; the excitonic molecule or bi-exciton and, given the right physical conditions, the condensed exciton system—the Bose droplet. The latter has been detected in sizes up to 1 mm as reported by C. D. Jeffries (University of California, Berkeley). Although such phases are not yet very evident in the organic systems, the spate of papers on exciton migration and interaction in aromatic crystals leaves no doubt that larger co-operative entities are likely to be found there also. The larger-than-usual collection of papers on organic crystals had a unifying influence on the meeting which should be strengthened in the future. There was also an important group of papers from P. M. Rentzepis (Bell Laboratories, New Jersey), R. Kopelman (University of Michigan) and V. J. Koester *et al* (Purdue University) which showed that picosecond and other spectroscopic-time resolution techniques can be applied to luminescence and related optical processes in chlorophylls with a good chance of unravelling the initial excitation and transfer mechanisms in photosynthesis.

The radiationless transitions which detract from luminescence efficiency are usually only indirectly observed through quenching of luminescence. M. B. Robin (Bell Laboratories, New Jersey) has, however, detected the heat pulse from a non-radiative transition by means of the 'second sound' pulse propagated in superfluid helium and recorded by a superconducting lead bolometer. In a different experiment at helium temperatures, N. Riehl (Technical University, München), has shown that it is possible to detect single phonon propagations in crystals by their activation of electrons from very shallow traps.

Among new luminescent materials reported were some stoichiometric rare earth compounds (such as NdP₂O₇) developed by M. G. Danielmeyer (Max-Planck-Institut für Festkörperforschung, Stuttgart) needing no impurity doping and having high efficiencies as

laser crystals. M. Genet *et al.* (Institut de Physique Nucléaire, Orsay) described a ThBr₃ phosphor, efficient as a scintillator and capable of excitation by a β source to give enough light to a photovoltaic cell to provide an energy converter of about 0.3% efficiency! To these we may add recent phosphors, reviewed by A. L. N. Stevels (Philips Research Laboratories, Eindhoven) which may be used to give three component fluorescent lamps, substitutions for the sulphide phosphors of cathode ray tubes and for the current X-ray intensifying screens. All of these materials utilise the features of rare earth activators in a variety of host crystals.

Among new effects reported at the meeting, E. Nakazawa (NHK Broadcasting Science Research Laboratories, Tokyo) described photon absorption and emission at twice the energy of the individual level separations of cooperating pairs of rare earth ions in an yttrium phosphate crystal. One might ask, what has happened to the former extensive interest in electroluminescence? There were some papers on the subject at the meeting but lack of financial largesse has forced research into a narrower range of materials, notably into studies of the deep lying band gap states in GaP, GaAs and their alloys and into other features which might be holding down the efficiency of light-emitting diodes. This basic work seems preferable to free ranging over every possible electroluminescent system although there is still an unsatisfied hope of a breakthrough in high band gap crystals like ZnS and ZnSe.

The institution of informal discussion groups on specific topics, such as picosecond techniques, proved very popular. Organisation in all respects was excellent and set a high standard for emulation at the next conference to be held in Paris in 1978. □

Neutron diffraction in Holland

from G. E. Bacon

A meeting on New Methods and Techniques in Neutron Diffraction was held on August 5-6 at the Reactor Centrum Nederland near Amsterdam. Papers presented at the meeting will be published as a report of the Reactor Centrum Nederland.

AFTER attending international meetings on neutron diffraction for a quarter of a century it was a new experience to go to one at which only 8, out of an

audience of 127, came from the United States. At first sight such a circumstance at the Reactor Centrum Nederland at Petten, near Amsterdam, may be thought simply to underline the increasing financial difficulties which face those who have to travel large distances to keep in touch with their scientific colleagues. More fundamentally the unusual ratio reflected the dividend which is now being paid for the Franco-German, and latterly and more tardily the British, collaboration in the High Flux Beam Reactor at the Institut Laue-Langevin in Grenoble. More specifically, much of the discussion at this meeting reflected the success of the German investment in guide tubes and other neutron optical devices inspired by H. Maier-Leibnitz. The papers were restricted to topics relating to elastic scattering only.

The six sessions made it clear that the number of available techniques in this field is immensely larger than it was 5 or 10 years ago, mainly because a high flux makes a practical possibility of many methods which developments in computing had made possible, in principle, some years earlier. The employment of position-sensitive detectors, contrast-variation in small-angle scattering by biological substances, polarisation analysis and powder-profile refinement are now producing scientific results concerning interesting materials, rather than demonstration experiments. For example, in protein molecules, by labelling different parts of the molecule with selective deuteration, it is possible to do much more than determine an average radius of gyration. By small-angle scattering also (G. Lippmann, Institut für Festkörperforschung der Kernforschungsanlage, Jülich, BDR) the dimensions of the nickel-depleted zones in invar alloys have been found. By profile-refinement, structures have been determined (J. C. Taylor, Chemical Physics Division, Lucas Heights, Australia) for a wide range of uranium compounds which are very intractable for growing and maintaining as single crystals. D. Hohlwein (Institut Laue-Langevin, Grenoble) indicated the practical possibilities of photographic detection, showing, for example, neutron Weissenberg photographs of the superstructure reflections from the mineral labradorite. M. Schlenker (Laboratoire du Magnétisme, CNR, Grenoble) showed how the penetrating ability of neutrons enabled topographic studies to be extended to the interior of crystals, thus effectively studying a section through a crystal without the disadvantage of having to destroy it by cutting a slice.

Rather belatedly perhaps, work has thrived on the use of pulsed sources, including particle-accelerating machines, for neutron diffraction experi-

ments using time-of-flight techniques. This is not solely because of the advantages which the fixed geometry yields for the production of cryostats, furnaces and high-pressure cells but probably with an eye to the future, where the next generation of neutron sources may well not be the conventional reactor, but some kind of neutron booster or spallation machine capable of increasing neutron beam intensities by another order of magnitude. □

From babble to Babel

by Miranda Robertson

The Third International Child Language Symposium was held in London on September 3-5.

It was linguistic theory, and in particular the transformational grammar of Noam Chomsky, that led to the recent surge of interest in child language; but while the interest is unabated, it seems that the influence of linguistics has begun to wane. Chomsky's view that the deep structures of his transformational grammar reflect the innate organisation of the neural substrates for language led researchers to look for evidence in the earliest utterances of infants and in fundamental similarities in the first sentences of children speaking widely different tongues. It is now generally felt however that transformational grammar has failed to illuminate either.

Richard Cromer (MRC Child Development Unit, London), as chairman of the session on the acquisition of syntax, attempted to categorise the advances and retreats. For example, as he pointed out, there is less work on syntax but more on semantics. That is partly because of a belated realisation that it is impossible to separate the two, since in order to look at how a child starts to use syntax to express his meaning it is necessary to know what meaning he intended to express. And it is the need to understand the child's semantic intentions that has led to another important reorientation (or in Cromer's terms, an advance)—from the dogmatic to the pragmatic approach. That has led to the use of the ethological research strategy promulgated perhaps most notably by Professor Jerome Bruner (Oxford University).

What, then, have psycholinguists learned by watching the emergence of infant speech in its natural habitat? Elizabeth Bates and her colleagues (University of Rome) have been able to follow the progression from gesture to first word in a longitudinal study on Italian children from the age of 2

months. It is commonly accepted that the single-word utterances of infants can be divided into imperative and declarative modes, at a stage at which differences in intonation and gesticulation have to substitute for syntax to indicate whether the intention is to demand something or merely to point it out. Bates has been able to trace the development from imperative and declarative gesture alone to the later association with words.

As any mother knows, and as Linda Ferrier (University of Bristol)—who is one—pointed out, the meanings of babies' first words are frequently complex and usually idiosyncratic, because the child has to encode in one word the semantic content of a sentence and he generally does so by presuming on his shared experience with his mother. For example, Ferrier's own child used the word "cat" to request a drawing, because cats were what Ferrier habitually drew.

Because the single words of small children are so loaded with significance, they have come to be known in the jargon of the field as holophrases, whose interpretation is central to an understanding of how children develop more complex linguistic skills. Dr Patricia Greenfield (University of California, Los Angeles) therefore aroused particular interest with a report of preliminary observations on two children (one hers) on how a child selects one of several appropriate single words to utter in a given situation. Her thesis is that the decisive factor is the word's information content, somewhat intuitively defined in terms of novelty or unexpectedness. The child will thus draw attention to the aspect of a situation which surprises him and may be new to you; which seems a sound functional basis for communication but has no obvious linguistic implications.

Linguistic factors in the acquisition of speech proved generally elusive. Elizabeth Sharpless (City University of New York) found linguistic complexity accounted less well for the order of acquisition of the personal pronouns than did the part played by the child in her experiment. The crucial factor was whether he was a participant in the dialogue, or an onlooker.

H. H. Chipman (Geneva University), following the development of comprehension of possessive pronouns, remarked that at the earlier stages the child's interpretation of a sentence is quite independent of pronoun reference and seems to be based entirely on his presuppositions about the world. That sort of work exposes the need to consider a child's linguistic development in the light of his cognitive development in general, and David Ingram (University of British Columbia) and Bates and

her colleagues have been attempting a systematic study of the relationship between linguistic development and Piagetian cognitive stages. That has involved both of them in some redefinition of one or two of the stages but does not threaten Piaget's view that language develops as part of the child's general capacity for symbolic expression.

Comparisons on the speech of children of different nations, too, yield cognitive rather than linguistic universals, according to D. I. Slobin (University of California, Berkeley), who spoke on his own work and that of F. Antinucci (CNR, Rome) on children speaking Italian, Serbo-Croatian and Turkish. In general, the syntactic errors common to all children can be traced to cognitive problems inherent in what they have to express. Purely linguistic difficulties vary between languages so that, for example, Yugoslavian children are slower to grasp inflection, which is complex in Serbo-Croatian, but quicker than Turkish children to grasp relative clause structure, which is particularly difficult in Turkish. All this raises implicitly the question Cromer made explicit at the conclusion of his assessment of the field: should language learning be considered separately from other kinds of learning? He did not venture an answer, but on the basis of evidence presented at the symposium it should be no.

A. Buffery (University of London) presented a review of recent anatomical and psychophysical evidence for genetically determined asymmetry in the structure and function of the human brain which was widely regarded as evidence for the innateness and the uniqueness of linguistic abilities. But the anatomical evidence rests on an enlargement of part of the left temporal lobe in about 60% of neonates; and there is psychophysical evidence for functional asymmetry in visuo-spatial as well as linguistic skills. It may be legitimate to conclude that man is innately endowed with some elaboration of the cerebral cortex that enables him to use language, but not that it is qualitatively different from whatever allows him to do arithmetic, for instance.

It is beginning to seem that it may be a mistake to investigate the acquisition of language *per se*; and it should perhaps not be very surprising that philosophical linguistics have in the end relatively little to contribute. For one thing, language as spoken to and by children is very different from the sophisticated instrument it becomes in the hands of philosophical linguists. Roger Brown's frequently quoted point, that it was unlikely that a child could ever learn to talk from listening to the speakers at a learned conference, was

well taken, and several speakers addressed themselves to the question of how adults (and especially mothers) adjust their speech to talk to children.

Perhaps one of the most striking differences between children's speech and that of adult academics is that while the child's speech is generally strictly functional, the academic's is often counter-functional. Children thus use their limited lexical and syntactic resources to make their meaning clear, whereas academics may devote all the riches of theirs to obscuring it. Examples were not absent from the papers delivered at the symposium. □

Cosmic rays at Munich

from a Correspondent

The 14th International Cosmic Ray Conference was held at Munich on August 15-29.

A CLAIM for the detection of a new particle or interaction process seems to have become a regular feature at successive biennial cosmic ray conferences. This year it was the turn of the Monopole. In a specially arranged lecture, P. B. Price (University of California, Berkeley) presented the facts to a sceptical audience of some 500 cosmic ray physicists. The evidence is the track of a particle passing through a balloon-borne stack of Cerenkov film, nuclear emulsion and lexan sheets. The researchers (P. B. Price, E. K. Shirk, W. Z. Osborne and L. S. Pirskey) argue that in a stack of 33 lexan sheets the particle produced a track expected of either a nucleus with $125 \leq Z \leq 137$ and velocity $\geq 0.92c$ or a magnetic monopole with a magnetic charge $= 137e$. The Cerenkov/emulsion data indicated that the particle was moving downwards with $v/c = 0.5 \pm 0.1$, and was either a nucleus with $Z \sim 80$ or a monopole of charge $137e$. The only common explanation is hence the monopole. The evidence looked good to a critical audience.

Subsequent discussions, both in and out of session, produced one major attack on Price's conclusion. This came from P. H. Fowler (University of Bristol) who argued an alternative explanation in terms of a nucleus of charge ~ 82 interacting twice in the lexan stack and losing three units of charge at each interaction. By the end of the fortnight it was difficult to find many people who were highly convinced by Price's arguments. The most popular attitude was to wait and see.

The cosmic ray field today covers a vast range of topics 'from quasars to

quarks' and this was reflected in the more than 800 original papers presented at the conference.

On the more traditional cosmic ray stage Price and Shirk also presented data on the distribution of heavy nuclei ($Z > 65$) in cosmic rays obtained from a 1.3 m² lexan detector aboard Skylab. These researchers have obtained data with very high charge resolution and conclude that the measured charge distribution in the vicinity of the Earth at ~ 0.5 GeV per nucleon strongly favours synthesis of these cosmic rays by the r-process (rapid neutron capture) within the last 10^7 yr.

Measurements of cosmic ray particles at very high energies ($> 10^{17}$ eV per nucleus) are made indirectly by means of the extensive air showers (EAS) produced in the atmosphere. Conclusions on the composition of the particles at these energies are still far from definite. The energy spectrum of the primary particles is however, now becoming well established up to 10^{20} eV per nucleus. Further analysis presented by the University of Leeds group, using data obtained at the Haverah Park EAS detector array, gives added weight to the evidence against there being a cut-off in the energy spectrum $\sim 5 \times 10^{19}$ eV. Such a cut-off has been predicted to arise from the interaction of the microwave background radiation (2.7 K) and the cosmic ray particles. The absence of a cut-off seems to be inconsistent with the universal abundance of both the microwave photons and the high energy cosmic ray particles.

Moreover, strong evidence seems to be accumulating for a distinct anisotropy in the celestial arrival direction of the highest energy cosmic rays ($\sim 10^{19}$ eV). As A. A. Watson (University of Leeds) emphasised, the two highest energy events recorded at Haverah Park ($> 10^{20}$ eV) both come from directions close to the North Galactic Pole—strongly indicating a non-Galactic origin. Arrival direction analysis of 'muon-rich' EAS (Nottingham University group) gives indication that heavy cosmic ray primaries at lower energies (10^{17} – 10^{18} eV) may also show anisotropy in a similar direction.

From both interest and necessity cosmic ray physicists have to concern themselves with high energy nuclear physics as well as astrophysics. Previous claims for the detection of tachyons and mandelas (heavy, long interaction length particles) in cosmic rays have unfortunately had to be withdrawn at this conference. Moreover none of the recent searches for fractionally charged quarks amongst the secondaries of cosmic rays have proved positive. Other new phenomena still do seem to be present at these energies, however, most notably the X particles discovered

by the Japanese emulsion group. Also it seems to be increasingly difficult to reconcile EAS data with the sealing model of nuclear interactions. \square

The breakdown of physics?

from Malcolm MacCallum

A Relativity Workshop was held at Gregynog Hall of the University of Wales on September 1–3.

"God not only plays dice. He also sometimes throws the dice where they cannot be seen." This statement is made by S. W. Hawking (Cambridge University) in his recent work on black holes. About eighteen months ago he demonstrated that quantum processes could lead to the emission of thermal radiation from black holes, so evaporating them. This showed that the formal analogies between entropy and the area of the hole, and temperature and the surface gravity of the hole, are genuine identities. Now Hawking has circulated two preprints which develop this argument, which were discussed along with related papers at the workshop.

In the first he discusses the thermodynamics of black holes. He interprets the meaning of the entropy in terms of lost information about the initial state, proves that the second law of thermodynamics holds, and proceeds to argue, by considering a black hole in a box, that as a consequence of time-reversibility and ergodicity the emission from white holes (the time-reverses of black holes) must be thermal, and that hence black and white holes are indistinguishable.

In the second—"Fundamental Breakdown of Physics in Gravitational Collapse"—Hawking proves that the radiation has thermal statistics as well as spectrum, in agreement with independent work of L. Parker (University of Wisconsin) and R. M. Wald (University of Chicago). He argues that the random loss of information into the black hole implies the breakdown of S-matrix theory and that a density matrix formulation is all one can give. By considering further the white hole in a box he infers a 'Randomicity Principle' that singularities emit all possible configurations with equal probabilities, as pithily described above. Hawking asserts that this is necessitated by CPT invariance or by the non-existence of perpetual motion machines.

B. F. Schutz (University College, Cardiff), the organiser, opening the conference, pointed out that these two papers re-established the primacy of the thermodynamics. Various speakers emphasised the heavy dependence of

Hawking's arguments on the full applicability of standard thermodynamic results such as the meaning of entropy and the ergodicity theorem, and questioned the correctness of these assumptions. Schutz pointed out that application of Hawking's black hole calculations to white holes led to a burst of radiation with Rayleigh-Jeans spectrum at infinite temperature, resulting from particle creation from an initial vacuum. This might upset the thermal nature of any emission from within the hole itself. Schutz suggested some ways out of this difficulty, including the existence of a remnant black hole after the white hole explosion. C. J. S. Clarke (University of York) similarly suggested that a black hole evaporation must leave behind a naked singularity.

M. Perry (Cambridge University) presented some work on the modification of Hawking's picture to allow for strong interactions between the particles produced at high temperatures. Unless a very soft equation of state is used, the only consistent picture is that this is unimportant.

R. Penrose (Oxford University) expressed disquiet with the idea of black holes in boxes re-forming by the time-reverse of the evaporation process, which is important to Hawking's argument about equivalence with white holes. He also offered a modification of Hawking's proposed fluctuation mechanism for temporary destruction of the black hole, and various participants did back-of-envelope calculations to substantiate his point.

S. Fulling (King's College, London) and P. Candelas (Oxford University) gave talks on the technical aspects of quantum field theory in curved space-time, focusing on the definitions of the creation and annihilation operators and the vacuum state, and on the Feynman propagator. These talks explored, *inter alia*, the fact that incautious use of Rindler coordinates in flat space produces results inequivalent to the usual quantisation. Fulling showed that the treatment of the Hawking mechanism due to D. G. Boulware (University of Washington), which predicted no radiation, has failings of this kind.

The meeting was highly informal and many short contributions were made in discussion. The consensus seemed to be that the broad sweep of Hawking's ideas involved a great many interesting points, whose further examination was necessary. This will be an extensive programme since conceptual problems in general relativity, in thermodynamics and in quantum theory and their relation with general relativity, as well as difficult calculations, will be involved. The meeting helped greatly in clarifying some of the questions to be studied. \square

review article

Contacts with semi-insulators

H. K. Henisch* & C. Popescu†

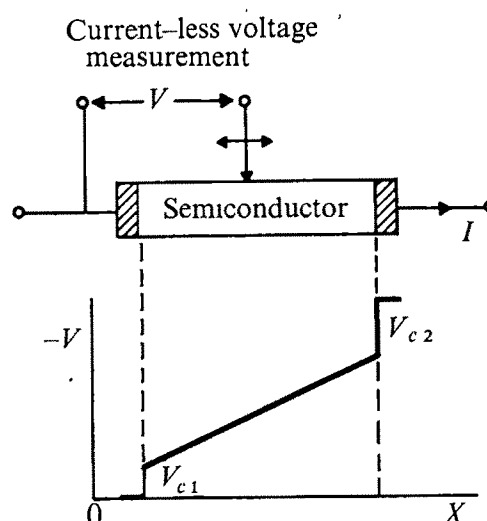
Semiconductor contacts are of considerable practical interest because of their uses in rectification and photovoltaic energy conversion. Recently, however, they have become the focus of the critical controversy because of some unexpected behaviour on the part of contacts associated with semi-insulators.

SEMICONDUCTORS are believed to owe their very discovery to the observation of contact effects in the 1830s. The subject of semiconductor contacts has been of considerable interest ever since, often because contact properties themselves can be very useful, for example in the contexts of rectification and photovoltaic energy conversion¹. In such cases one aims to separate the contact properties from bulk (volume) properties, and to suppress the latter. At other times, when it is the bulk properties that one wishes to select, it turns out that most of these are experimentally "inaccessible" without the use of contacts in one form or another. The suppression of contact effects then becomes the principal aim but this is not necessarily a simple matter, nor indeed is there complete clarity on the criteria for success. Thus, whether wanted or unwanted, contacts (which must be regarded as abrupt heterojunctions) deserve attention and study. They have received a great deal of both in the past, chiefly in the context of technologically important semiconductors such as Ge and Si. Much less is known about effects associated with contacts to semi-insulators, that is materials with resistivities of the order of (say) 10^4 – 10^{10} Ω cm. This is now an active field of study in the context of which new insights have recently been gained.

All precise knowledge of contact effects begins, in principle, with an experiment of the kind shown in Fig. 1. By means of current-less potential probes, whether fixed or movable, the potential drop along a semiconducting bar is explored and compared with the potential difference across the two-terminal system as a whole. This procedure reveals the existence of voltage drops 'at' the contacts, which depend asymmetrically on the current density, as shown in Fig. 2. When this was discovered (on semiconducting sulphides) in the 1870s, it was realised that voltage drops cannot really occur at the interface planes between contacting materials, but must be across some regions of the specimen. Foreign contaminants suggested themselves as a source of the additional resistance, but none could be compellingly identified in that role. It became clear that if some "special region" were involved, it had to be made up of the parent material itself, though modified in some way to make it near-insulating. The explanation of the mystery came with the development of the Mott-Schottky potential barrier models of the 1938–42 period^{2,3}. These models explained not only the voltage drop at contacts but also the asymmetrical departures from Ohm's law associated with them (Fig. 2). These departures result from the presence of carrier concentration gradients which, in turn, imply diffusion currents, to which Ohm's law is in no way applicable. The departures from linearity

are asymmetrical, because the contact barrier is asymmetrically distorted by externally applied voltages, as shown schematically in Fig. 3. Typical barrier thicknesses range from 10^{-6} to 10^{-4} cm, depending on the nature of the surface and the purity of the semiconductor. For an appropriately simplified single-carrier system the carrier transport equation was easily solved, and a voltage-current relationship calculated. Refinements were later introduced to take account of image forces and hot carrier effects, but the model is still used today substantially in its original form. Whether it should be so used is another matter. Schottky himself pointed out that the barrier actually contains too few discrete (and, by assumption, randomly distributed) centres for the space charge to be treated as continuous, and an appropriate correction for this fact has never been devised. Moreover, the model assumes barrier heights greatly in excess of kT and thus applies only to high barriers. Similarly, its validity is limited to thick barriers, for which tunnelling processes can be ruled out. The last two assumptions limit the validity of the model to barriers of high resistance, and whereas no limitation is actually welcome, this particular one is less damaging than one might think because, in practice, we know so much more about contacts of high than of low resistance. This is so because very high voltages are not ordinarily applied to low resistance contacts, and under low voltages (that is voltages smaller than kT/e) all contacts behave almost linearly, as is obvious from Fig. 2. Such contacts may appear "ohmic" for practical purposes, but this does not mean that they are free from barriers (or carrier concentration gradients arising from other causes). Barriers may in fact be present, and would undoubtedly show

Fig. 1 Determination of contact voltages.



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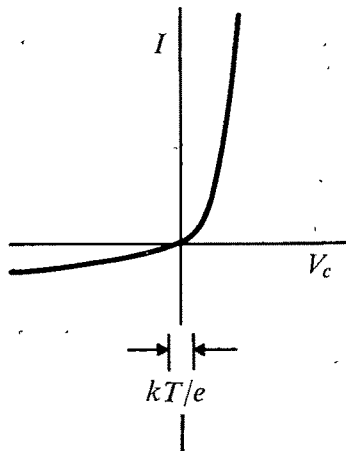


Fig. 2 V - I characteristic of metal-semiconductor contact.

themselves if higher voltages were applied. Such an experiment would, however, have to be carried out with very short voltage pulses, to prevent destruction of the system by heat. Contrary to popular belief, there is no simple relationship between the magnitude of a contact resistance and its linearity. Whether a contact is "ohmic" or not, that is whether it conforms to Ohm's law, thus becomes a relative assessment, and that is actually the only sense in which the term "ohmic" can be safely and unambiguously used.

Because of the experimental difficulties involved, low resistance (low barrier) contacts are rarely studied in a quantitative way. Moreover, they cannot be theoretically handled by the classical transport theory, but call for wave mechanical treatments along the lines of the Fowler-Nordheim analysis, modified by space charge considerations. Such treatments had been completely lacking, until the work of Gossick^{4,5}. "High" and "low" barriers are obviously limiting abstractions as, indeed, are "thick" and "thin" barriers, especially when we are dealing with non-rectangular energy contours of variable shape. The most general case is therefore highly complex and awaits the attention of some dedicated worker with an unflinching enthusiasm for modern computer techniques.

The above account assumes that contact and bulk resistances can always be clearly distinguished from one another, for example by an experiment of the kind shown in Fig. 1. This is equivalent to the assumption that the semiconductor material under a contact remains electronically unchanged by the presence of the current. It was precisely the breakdown of this assumption that led to the invention of the transistor. Bardeen and Bratton⁶ were exploring the field pattern in the neighbourhood of a point contact which was passing a current in the forward direction. They found that the material to a substantial depth under the contact behaved as if it had higher than normal

bulk conductivity. Additional charge carriers were evidently present in the affected region, but charge carriers of one sign could not be contemplated, because the resulting space charges would have been enormous. The effect could not, therefore, be explained on the basis of a single-carrier model, and in response to this difficulty the inventors of the transistor formulated the concept of minority carrier injection. The contention (since then amply confirmed) was that, at the contact and immediately below, the current is carried not by the majority carriers which predominate in the bulk, but by minority carriers. In n -type material, the injected carriers would thus be holes, present in (non-equilibrium) excess concentrations ΔP . These carriers drift and diffuse in the semiconductor as far as their "lifetime" will allow. Of course, their contribution to the current cannot actually "disappear"; in due course injected minority carriers recombine with resident majority carriers in a manner which ensures current continuity. Before the minority carriers have time to recombine, they attract (in media of relatively high conductivity) an excess ΔN of charge-compensating majority carriers into the injection region. The result is an excess of both types of carriers in a region which remains essentially neutral, and this accounts for the higher than normal conductivity. Figure 5a shows this situation. The magnitude of the increments ΔN and ΔP depends on the carrier lifetime, on the height of the contact barrier (relative to the band gap), and on the current density.

In due course it was found that minority carrier injection is only one out of four possible non-equilibrium processes which can be sustained by currents through semiconductor contacts, and are controlled by minority carriers. The others are minority carrier accumulation, exclusion (terms originally coined by P. C. Banbury) and extraction (see Fig. 4). For reasons which are outside the scope of this discussion, only one of these four effects, namely injection, has so far been shown to lend itself to practical applications, and that is why we have (in that sense) only one type of transistor. For the same reason, minority carrier injection is the only process considered here. In specific contexts, the non-equilibrium effects can be negligible, but that a contact should exhibit none of the four effects in greater or lesser degree is actually inconceivable. In the presence of a current, any discontinuity in the conductive properties must lead to the formation of a transition region, within which the carrier concentrations depart from equilibrium, and the current composition adjusts itself to the normal bulk mode. Thus, every contact is associated with contact effects, and we have learned a good deal (if not actually enough) about the design of contacts with specific properties.

Until recently, it was always assumed that the above non-equilibrium effects would be totally suppressed in materials of very short lifetime, but even this apparently self-evident expectation involves an internal conflict. Implicit in the above account is the assumption that the carrier lifetime τ_0 in the semiconductor is much greater than the dielectric relaxation time τ_D ("lifetime semiconductor"). It is this assumption which permits

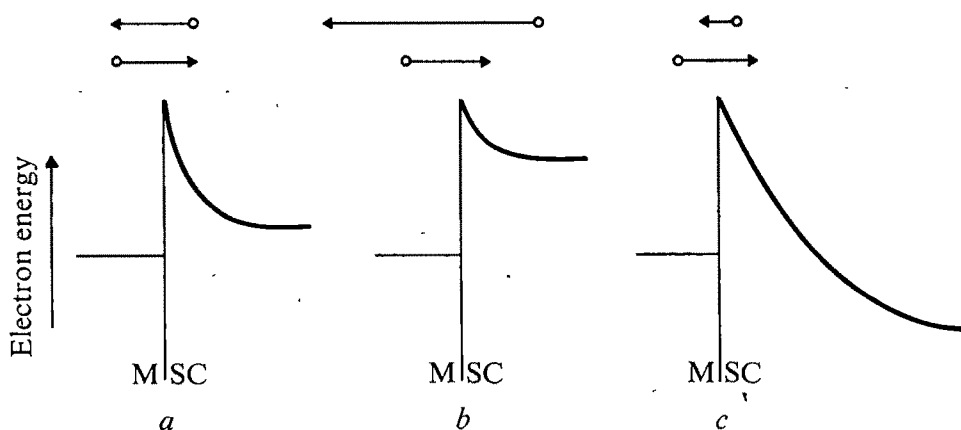


Fig. 3 Rectification through asymmetric distortion of the contact barrier (shown for n -type material). *a*, In equilibrium; *b*, with a forward voltage applied; *c*, with a reverse voltage applied. Arrows denote electron flow. M, metal; SC, semiconductor.

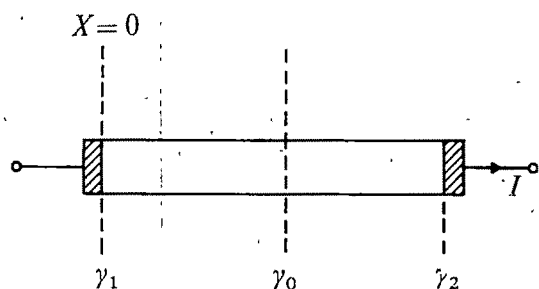


Fig. 4 The four non-equilibrium effects which can result from current flow through a contact. n-Type semiconductor $\gamma_1 > \gamma_0$ injection; $\gamma_1 < \gamma_0$ exclusion; $\gamma_2 > \gamma_0$ extraction; $\gamma_2 < \gamma_0$ accumulation, where γ_0 is current composition rate. γ_0 (its value far from contacts) $= I_p/I$, where I_p = current carried by minority carriers.

minority carrier space charges to be neutralised by an influx of majority carriers, before carriers recombine. As long as $\tau_D \ll \tau_0$, the supply of compensating charges is always plentiful. If the lifetime τ_0 were to approach zero, however, this inequality could not be maintained, even in the relatively well conducting transistor materials. In materials of higher resistivity the situation is generally reversed, that is $\tau_D \gg \tau_0$, and neutralisation becomes very difficult.

The changes which the assumption $\tau_D > \tau_0$ implies in our picture of contact injection were not appreciated until recent years, when attention was drawn to them by van Roosbroeck and Casey⁷⁻⁹. In so doing, they opened a completely new field of inquiry. In particular, van Roosbroeck pointed out that minority carrier injection into a semi-insulator (a "relaxation semiconductor") would lead to majority carrier depletion ($\Delta n < 0$), and not to an excess of both carriers, as it does in the conventional lifetime semiconductor. In turn, the majority carrier depletion was expected to lead to a higher than normal total resistance, again in contrast to the lifetime case in which the total resistance of the system is lowered by injection. It was also predicted that the current-voltage relationship of such a system would be sublinear, and that the depletion region would spread throughout most or all of the available material. This spread was expected to have a peculiar and important consequence: it would invalidate one of the most popular tests for contact effects, namely the proportionality between specimen resistance and specimen thickness (bearing in mind that measurements on semi-insulators cannot be performed in the manner suggested by Fig. 1, but only on two-terminal thin film systems, without potential probes). The model suggested that, in relaxation semiconductors, this proportionality would be essentially

maintained in spite of the presence of minority carrier injection. The resistance determined in this way would, however, be a good deal higher than that calculated from the dimensions and the undisturbed bulk resistivity, and to that extent a totally misleading result could be obtained.

Of course, majority carrier depletion can be expected to have an important role only as long as the material contains a significant number of majority carriers to deplete. In really wide-gap insulators, there are virtually none, and all injection (whether of majority or minority carriers) then gives rise to purely space charge controlled conduction processes, of the kind analysed and discussed by Lampert and Mark⁹. These processes give rise to a proportionality between (current) and (voltage)², equivalent to Child's law in a vacuum, but different because of the limited mean free path of carriers in a solid. In what follows, we are concerned with poorly conducting materials in which, however, resident carriers still play an important part.

The startling van Roosbroeck predictions were duly challenged in the literature, and even the notion of majority carrier depletion as such was thrown into serious doubt. One of the difficulties is that the two-carrier transport equations cannot be explicitly solved. "Simplifying assumptions" can certainly be introduced, but whether these distort the picture or are truly helpful can be reliably assessed only by comparison with the full solutions, and these must come from the computer. They have only recently become available (refs 10 and 11 and C.P. and H.K.H., unpublished), and their general significance may be outlined as follows.

The relationships can be best understood by comparison with the more familiar lifetime case. Figure 5 gives computed contours for the additional carrier concentrations ΔP and ΔN , as well as the field, as a function of distance from the injecting interface. Figure 5a and b are for the same normalised current density, the only difference being the ratio τ_D/τ_0 (or, somewhat more accurately, τ_{Dn}/τ_0 , where τ_{Dn} is the relaxation time arising from majority carriers alone). It will be seen that the majority carriers are indeed strongly depleted (ΔN negative) under the contact in the relaxation case, as van Roosbroeck had suggested. The disturbance extends over a number of Debye lengths which, in a semi-insulator, can amount to an appreciable depth. The depletion is virtually complete and, accordingly, the recombination of injected minority carriers is inhibited in this region. It therefore takes place in "recombination front" (where ΔN has its sharp gradient). It can be shown that the total carrier concentration $N+P$ is also depleted over a certain distance and has a well defined minimum. This minimum falls, however, within a region in which the diffusion currents are high, and in such a region carrier concentrations do not control the current; concentration gradients do. The minimum of $N+P$

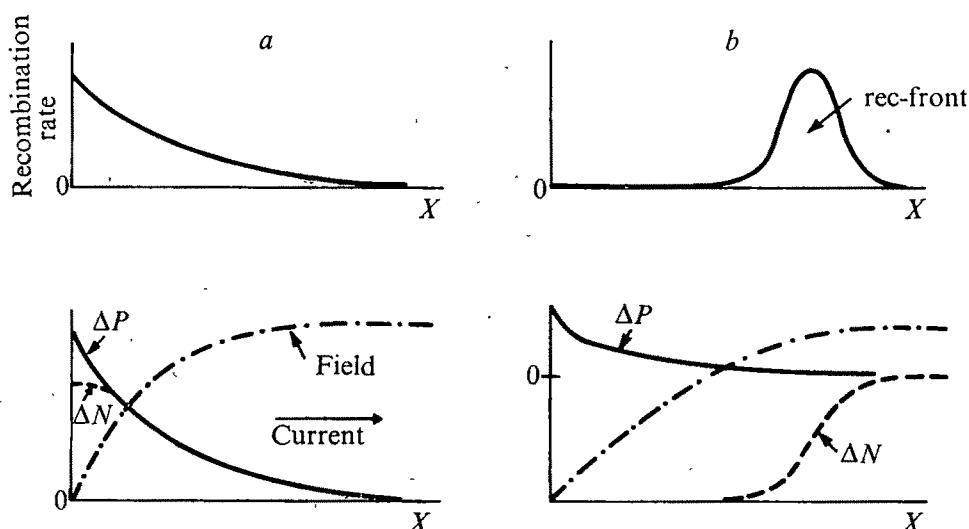


Fig. 5 Consequences of minority carrier injection into n-type material. a, Lifetime semiconductor ($\tau_D/\tau_0 \leq 1$); b, relaxation semiconductor ($\tau_{Dn}/\tau_0 \geq 1$), for same (normalised) current density.

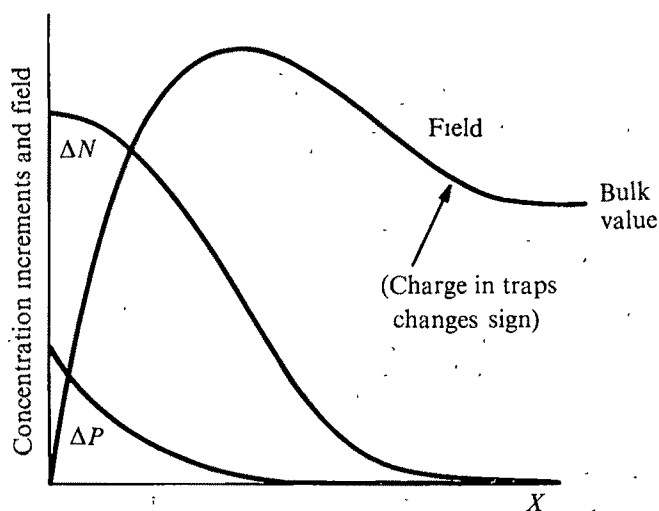


Fig. 6 Carrier concentration and field contours for minority carrier injection into a relaxation semiconductor containing traps.

does not, therefore, govern the resistance of the system as a whole. Assuming bimolecular recombination and equal electron and hole mobilities, the total resistance can be shown to be smaller than that expected from the bulk resistivity, in spite of the majority carrier depletion. The presence of diffusion currents contributes to this fact, but the physical picture is clear enough even without them. The resistance is actually determined by two opposing tendencies, of which the first wins: (1) the high concentration of injected minority carriers, and (2) the depletion of resident majority carriers.

It is interesting to consider the behaviour of such an injection system under illumination¹¹. In the presence of radiation, the space charge system is modified; accordingly, recovery (when the radiation ceases) involves not only the recombination of excess carriers but a space charge movement. This means that the time constant of photoconductive decay is not a pure carrier lifetime, but a complex mixture of recombination and relaxation effects, involving τ_D as well as τ_o . As a consequence, carrier lifetimes cannot ordinarily be determined from photoconductive decay experiments in the relaxation case. The calculations of Fig. 5 refer explicitly to the contacts of "unit injection ratio", that is contacts for which the entire current is made up of minority carriers at the metal-semiconductor interface. In terms of a semi-infinite n-type semiconductor, this means $J_o = J_{po} = J_{\infty}$, where J_{∞} , the current far from the contact, is, of course, carried by electrons. Quantitatively, the available results are limited to their case, but *mutatis mutandis*, the model applies also to lower injection ratios. Of course, all injection effects are then diminished.

If the above (idealised) model yields only lower than normal resistances, as it does, there remains the interesting question of whether higher than normal resistances can ever be envisaged, as originally suggested by van Roosbroeck. A search for such forms of behaviour must inevitably turn to systems of a more realistic (albeit more complicated) kind. To be realistic, a model must include some consideration of trapping processes, which are almost universally characteristic of semi-insulating and insulating materials. Trapping has two aspects: (1) it involves recombination through centres, implying a carrier lifetime which is not constant, as assumed above, but dependent on injection level, and (2) it involves space charge storage. Of these, the second is actually the more potent factor, as recent calculations (C.P. and H.K.H., unpublished) have shown. To understand the role of the traps, it is convenient to refer again to Fig. 5a, even though these particular contours apply to trap-free conditions in a normal lifetime semiconductor. It will be seen that over most of the injection region neutrality prevails, but close to $X = 0$ there is an imbalance, which creates an electric

field. This field actually serves two distinct purposes, both designed to provide electrons in sufficient numbers to satisfy the recombination needs: (1) the field supports electron drift towards the injecting plane, and (2) it opposes electron diffusion under the prevailing (negative) concentration gradient. Because of (2), the field is locally greater than that required to sustain the electron recombination current as such. The greater the concentration gradient, the greater would the field in this region have to be. These relationships are general, but they have a particularly important bearing on the case with traps.

In n-type material trapping states (assumed for simplicity to be located half way across the forbidden band and in equal communication with electrons and holes), would change their occupancy in response to any non-equilibrium situation; in other words, these traps could trap electrons or holes, depending on the relative concentrations. It is then an easy matter to show, on the basis of quite conventional Shockley-Read considerations, that the trap response to excess holes is much more sensitive than that to excess electrons; the traps are overwhelmingly hole traps (as long as there are holes to trap). A very small unbalance in the equilibrium trap occupancy is sufficient to produce enormous fields, and if there were no compensating mechanism, the positive space charge density would soon become quite excessive for the maintenance of current continuity. There is, however, a compensating mechanism, namely the attraction of majority carriers from the bulk material (Fig. 6). Accordingly, an excess ΔN of free electrons appears in the injection region. We then have $\Delta N > \Delta P$, the charge difference being made up (almost everywhere except close to $X = 0$) by the trapped (immobile) holes. This happens in the relaxation case as well as the lifetime case. Indeed the presence of traps can change the situation from a relaxation regime to a lifetime regime. The appropriate criterion is then no longer τ_{Dn}/τ_o , but $\tau_{Dn}/\tau_o (M_o + 1)$, where M_o is the (normalised) trap concentration. In the trap-free case, the field has its highest value in the bulk, as Fig. 5 shows, but when traps are present in sufficient concentration, the maximum field is in the disturbed region (Fig. 6). This means that the total resistance is indeed higher than that expected from the bulk resistivity, though for reasons very different than those originally envisaged by relaxation semiconductor models. The emphasis here is not on the fact that we are dealing with injection into a relaxation semiconductor but on the fact that we are dealing with injection into a material containing large numbers of traps. That is when the negative gradient of majority carriers appears, even when the carrier lifetime is shorter than the relaxation time. In otherwise comparable conditions, lifetime semiconductors can be shown to exhibit similar and, indeed, more pronounced resistance enhancement effects. In Fig. 6, the field is at its maximum necessarily where the concentration gradient is at its maximum (and not within a majority carrier depletion region). The general conditions of current continuity are maintained. Of course, high carrier lifetime coupled with high trap density do not ordinarily go together, but in good photoconductors they do. Moreover, recent observations on gallium arsenide along these lines by Ilegems and Queisser of Bell Telephone Laboratories may refer to just such a combination of parameters, and the internal resistance of many junction-type solar cells must depend on these considerations.

One of the interesting aspects of the matter is the way in which a challenge of long cherished assumptions has led to completely unforeseen forms of behaviour. Moreover, what has been done so far is only a beginning; virtually every kind of electric, photoelectric and galvanomagnetic measurement procedure applied to semi-insulators is due for re-examination, a process which will no doubt take several years, and which may lead to new applications as well as a new understanding of transport processes.

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articles

Limits to energy release and utilisation from chemical fuels

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New combustors effectively abolish limits of flammability and extend the concept of what is a fuel. By burning very lean mixtures they also minimise pollution and irretrievable energy losses during conversion. The theoretical saving is more than double the proportion currently contributed by all nuclear and other 'non-combustion' sources.

PREVIOUS communications^{1,2} re-examined combustion strategies in response to energy shortages and described one particular burner³ for mixtures of very low heat content. This was based on massive heat recirculation between products and reactants, without simultaneous dilution by recycling products. Quite small models enabled mixtures to be burnt which corresponded in heat content to only one-third of the normal "limit of flammability" and this at flow rates two orders of magnitude in excess of the burning velocity of limit mixtures.

In terms of the conventional definition of a fuel as "something that will burn" such devices evidently extend the definition of "what is a fuel?" Quite apart from the various sources of very lean mixtures which could be burned by such methods³, (for example, ventilation air from mines, waste gases from various industrial and fermentation processes) many materials potentially capable of exothermic reaction are not now regarded as fuels because they contain a large proportion of inerts. Although brandy is generally recognised as a fuel in a combustion as well as a metabolic sense, beer, for example, would probably be considered so only in the latter sense. More seriously, methane diluted with 90% of inert gases would not previously have been rated as a fuel because it would not burn in air—that is, the stoichiometric mixture it would produce with air, if it were to burn as a diffusion flame, would lie below the normal limit of flammability.

In the majority of applications the question is not only "what can we burn?" but also "how much of the energy released can we usefully abstract?" There are, in fact, quite a few exceptions to this in the realm of the combustion of very lean mixtures because it is often desirable simply to burn up noxious pollutants to more innocuous products. In the present—and future—climate of energy shortages, however, the thermodynamic potential at which combustion energy can be utilised must always be a major consideration.

Since an improvement of energy utilisation from combustion of only 12.5% could, at present, replace all nuclear and other energy sources, we feel, generally, that insufficient attention is being paid to these problems. Accordingly, we have investigated

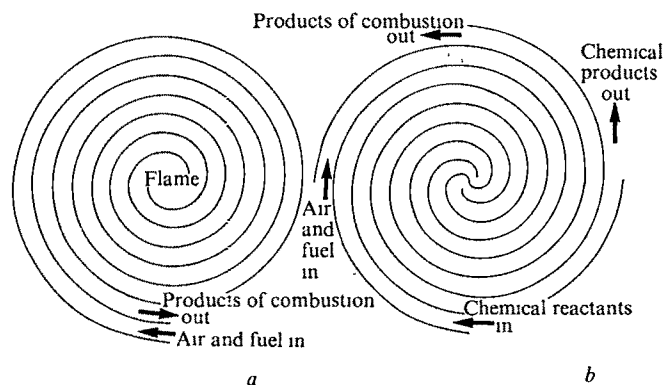
the absolute minimum heat contents at which stable burning can be maintained using the principle of recirculatory burning and the thermodynamics of conversion of the heat released from such lean mixtures.

Minimum heat content for combustion

The geometry of the "Swiss roll" burner³ is most readily appreciated by reference to a diagram of its cross-section (Fig. 1a). This is purely schematic: the depth (perpendicular to the diagram), the width and number of turns, the size, nature, and packing of the central combustion chamber, as well as the material of construction, are all variable. Since the maximum temperature attained in a combustion zone (see Fig. 2a) is determined by the amount of heat transfer, the reaction rate and thus the heat content of the 'minimum combustible' mixture are all functions of these geometric variables.

In the series of experiments designed to establish the leanest mixture which could be burnt, no heat was abstracted. The main sequence of experiments was carried out using methane as fuel, though a few tests were carried out on hydrogen, propane and ethyl alcohol. The burners were constructed of Inconel 600 strip, the width, number of turns of the spiral, and insulation being progressively increased to minimise heat losses as the fuel content was decreased. Thermocouples sealed into the spiral recorded the five temperatures in the positions shown in Fig. 2a; the initial, final, maximum just before and just after the combustor being denoted by subscripts 0, f, m, 1 and 2 respectively. Variation of flow velocity for each set of conditions produced

Fig. 1 Schematic of spiral cross section. a, Simple burner; b, second channel for processing another reactant.



stability loops similar to those observed previously³. If there were no heat losses, first law considerations demand that

$$T_m = T_1 + q = T_2 = T_0 + (T_2 - T_1) + q$$

where q is the temperature rise across the combustion zone, also equal to $(T_1 - T_0)$, and $(T_1 - T_0) = (T_2 - T_1)$. Absence of dissociation at these low temperatures, constant specific heat and a heat of reaction independent of temperature are assumed throughout for these highly diluted mixtures. In the present experiments there were always some heat losses (though it is possible to eliminate them almost completely as we show later) and errors in thermocouple readings include radiation exchange with the walls as well as catalysis, particularly for T_1 , at low fuel concentrations. Labelling heat losses as shown in Fig. 2a, the above equations become:

$$\begin{aligned} T_m &= T_1 + q - L_2 = T_2 + L_3 \\ &= T_0 + (T_2 - T_1) + q - (L_1 + L_2 + L_4) \end{aligned}$$

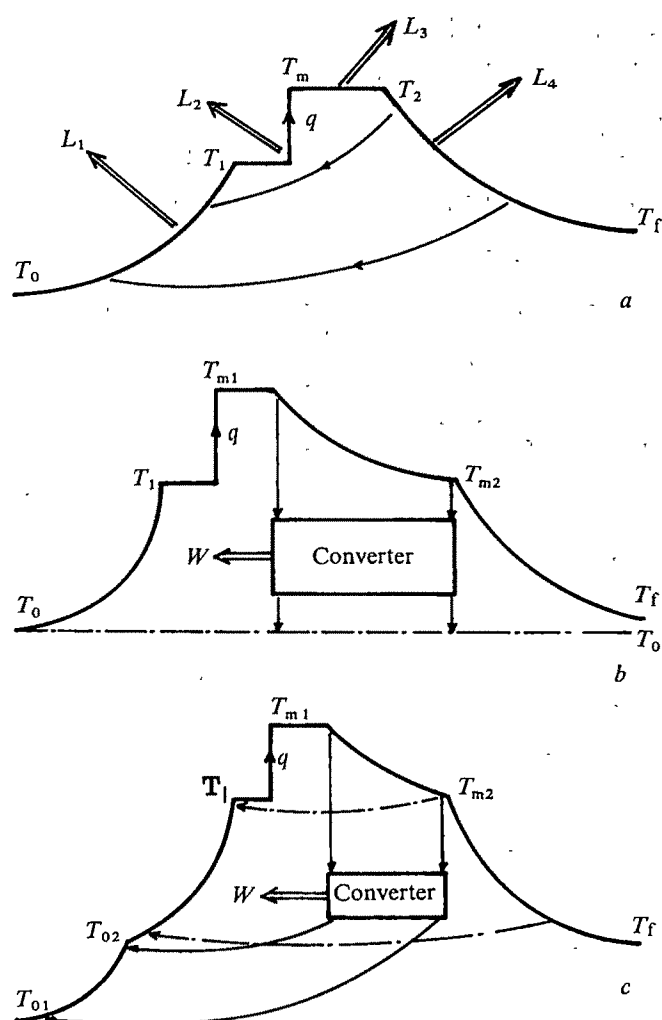
where

$$L_1 + L_2 + L_3 + L_4 = q - T_1 + T_0$$

and

$$L_1 + L_4 = (T_2 - T_1) - (T_1 - T_0)$$

Fig. 2 Temperature schemes without (a) and with (b,c) heat abstraction.



These, together with the measured values, were used to establish criteria governing stability limits.

The richest mixture studied was well below the flammability limit (5.3% methane in air) at 3.7% methane. Increasing the number of turns on the spiral for a given width of strip at first enabled leaner mixtures to be burnt. Plotting the leanest fuel concentration against the number of turns resulted in, approximately, rectangular hyperbolae—the decrease levelling off as sideways losses became controlling. Increasing the width of the strip and adding insulation caused the decrease in limiting fuel concentration to be resumed. We did not continue this progression to much below methane concentrations of 1%.

The salient feature which correlated the various temperature results (to be published in detail elsewhere) was extremely simple: the temperature in the reaction zone at the limit remained constant, within experimental error. For methane, this temperature is approximately 1,400 K. The explanation is thought to be that the limiting reaction rate is constant and, because of the high activation energy, very much more dependent on temperature than on composition. Using an overall activation energy⁷ of 268 kJ mol⁻¹, a change in the reactant concentration from 1.0 to 0.5% would correspond to a temperature rise of only 45 K.

Results obtained by Smith⁴ in a study of the temperature dependence of limits of flammability can be interpreted similarly. Although the mixtures were preheated by an external source and prereaction did not enable him to go leaner than 3.4% methane, the final flame temperature which can be calculated from the sum of his chemical and thermal enthalpies is in fact remarkably close to ours, at 1,430 K. Our much leaner mixtures burn without external intervention at much the same temperature and our extrapolation of this relationship would intersect the 'pure air' axis at about that temperature.

Having gone 82% of the way from the normal limit of flammability towards pure air, we discontinued the experiments and concluded that for all practical purposes there are no limits of flammability for combustion, when heat recirculation is not accompanied by simultaneous dilution of the reactants with products. In practice, sideways heat losses can be abolished altogether by turning the 'Swiss roll' into a torus. For materials which are not sufficiently flexible, or do not pass through a plastic stage during manufacture (as do some ceramics) an assembly of slices with non-parallel sides may be used. Such a configuration is of course not designed to withdraw heat at the highest temperature and, with a sufficient number of turns of the spiral, the product enthalpy flow is the only heat exit. In these conditions, however, anything capable of even marginally exothermic reactions becomes a potential fuel. The final temperature corresponding to the leanest mixture we burnt was approximately 225 K and, on the above activation energy argument, 0.22% methane should be possible before the temperature in the reaction zone would begin to damage our construction material.

Possible applications in which heat abstraction at the maximum temperature is not required include room heating, allowing the product to escape directly into the room. The saturated vapour pressure of conventional fuels is quite sufficient to enable air saturated with the fuel at room temperature to be burnt without previous atomisation or pumping and the combustion temperature ensures both complete combustion and negligible formation of CO and oxides of N₂. For burning up certain industrial waste gases and pollutants, the residual gas temperature may only be sufficient to provide enough buoyancy for dispersal through a stack. Among other proposals is the humidification, heating and sterilisation of hospital atmospheres by injecting a small proportion of hydrogen before passage through the burner.

Thermodynamic efficiency

When considering heat abstraction at the maximum temperature, for example, for conversion into other forms of energy, the conclusions are no less surprising than the finding that such

burners manifest effectively no limits of flammability. The striking conclusion is that, even starting from prime fuels, a higher conversion efficiency can result if the fuel is first diluted with a great excess of air.

There is a great variety of possible schemes for incorporating a converter, or heat engine, into the heat recycle. Figure 2b and c shows two examples: the former rejecting to an infinite (isothermal) heat sink and the latter to the heat exchanger inlet. A cursory treatment⁵ of the theory is somewhat inadequate and a more detailed account is being published elsewhere⁶. Although the overall efficiency is highest when the system shown in Fig. 2b is used with a thermodynamically efficient converter, for inefficient (for example, thermionic) converters it may be advantageous to use a different configuration. One possible reason is the large electrode area made available if parts of the spiral are used in that capacity³. For an inefficient converter, this advantage can outweigh the disadvantage of the rising temperature of the heat sink, particularly where the efficiency is overwhelmingly dependent on the temperature at which electrons are emitted⁶.

In this instance, however, we are concerned with the maximum amount (hence also pumping and other losses are neglected) of energy available. If we postulate converters consisting of a succession of elemental Carnot cycles the efficiencies corresponding to Fig. 2b and c can be shown⁵ to be

$$1 - [T_0/(T_{m1} - T_{m2})] \ln(T_{m1}/T_{m2})$$

and

$$1 - T_{02}/T_{m1} = 1 - T_{01}/T_{m2}$$

respectively. The nearest practical approach to these idealisations would be represented by two Stirling motors, their 'hot ends' contacting the sides of the combustion chamber in the centre of the 'Swiss Roll', their 'cold ends' being exposed to ambient conditions or, in the case shown in Fig. 2c, to a regenerator from which the reactants enter the spiral. The sides of the spiral would be insulated. In these conditions, the maximum value of temperature, T_{m1} , and thus of the efficiency, is entirely limited by the melting point of the confining material used and totally independent of the heat of reaction, since the heat recycle enables any temperature up to that maximum to be reached. (But for this restriction—and the effects of high temperature on dissociation and q —we could attain 100% efficiency.) As T_m tends towards infinity, the heat rejection tends towards zero. If we now allow the converter to use all the heat of reaction—that is, if we make the heat exchanger long enough to transfer the required amount of heat on a temperature differential which is negligible by comparison with that across the reaction zone—the two expressions for efficiency become

$$1 - (T_0/q) \ln[T_m/(T_m - q)] \text{ and } 1 - T_0/(T_m - q).$$

It follows that the efficiency increases as the temperature rise across the combustion zone falls, attaining the Carnot value $1 - T_0/T_m$ as q tends towards zero. The initially surprising result that efficiency can actually be improved by diluting the fuel, and making up the maximum temperature by recirculation, is simply due to the smaller temperature fall during the abstraction of the heat of combustion. The trend to the Carnot value in the first efficiency expression becomes more obvious on writing $\ln[T_m/(T_m - q)]$ as $\ln[1 + q/(T_m - q)]$ and expanding the logarithm for small values of q , whereupon the first expression reduces to the second.

The startling consequences become apparent on introducing realistic numerical values. Taking the maximum temperature at which the converter walls would melt, or oxidise, as the modest value of 1,500 K, the Carnot efficiency to which the above expressions tend at increasing dilution, is 80%. For the leanest mixtures burnt in the present work, $q/(T_m - q) \approx 0.18$. The corresponding efficiencies for the two systems are as high

as 78.3% and 76.5%, respectively. Using a richer mixture—such as would produce a final flame temperature of 1,500 K without recirculation—would give a maximum efficiency (external heat sink) of less than 60%. Thus the gain obtained from dilution and recirculation is equivalent to burning approximately one-third more fuel. On a global scale, this would provide more than double the requirement for replacing all nuclear contributions. Yet it takes no account of the difficulty of stabilising a flame of even that final temperature by conventional means. 1,500 K would be considered a very low flame temperature and it is the need for flame stability which is generally responsible for our using near-stoichiometric mixtures whose product gases, having generated oxides of nitrogen at these high temperatures, then have to be cooled down before they become acceptable to the converter.



Fig. 3 Radiant emission at maximum temperature.

This argument clearly applies only to systems limited by the melting point of walls. Where the hot gas is expanded while confined by cooled surfaces, other limitations, due to quenching, and so on, apply. As 1,500 K already corresponds to a Carnot efficiency of 80%, comparison with efficiencies attained in practice may cause us to reconsider the need for high combustion temperatures.

Similar considerations apply to abstracting heat at the maximum temperature, T_m , for purposes other than energy conversion (as distinct from using only the enthalpy of the product gases, at T_f , which we discussed earlier). For heat transfer by radiation, for example, using the temperature at the top of the 'thermal dam' is essential. Figure 3 illustrates this for a system fitted with a quartz window, the beam being made visible by scattering from smoke introduced in the vicinity and by the use of a 'luminous mantle' in the combustion chamber to increase the visible component of the radiation.

Figure 1b shows a four start spiral which was constructed for processing a fluid at the high temperature. Of the two neighbouring channels, one carries the combustion gases as before and the other the gas which, for example, undergoes an endothermic reaction at the high temperature. This may be used for the production of a higher grade fuel from the heat released by a lean mixture. The endothermic water-gas reaction, or reforming, provide simple examples of producing a higher specific calorific value from a low one and retrieving the unused thermal

energy. Although the heat flows are coupled, the mass flow rates in the two streams are of course entirely independent.

In the processing of solids, conveyance along spiral channels would be difficult and a more conventional furnace geometry is obtained by passing the furnace tunnel axially through a deep spiral. So long as heat carried away by the material processed and lost by conduction along the refractory tube is minimised, the essential principle of decoupling the heat flow from that of the product gases (which again leave through the spiral) can be maintained. Solid and liquid fuels which pyrolyse pose a similar problem. In this instance the most versatile arrangement is based on a fluidised bed in the combustion chamber, the surrounding spiral carrying only the incoming air and the departing product gases, the fuel being fed directly into the combustor. We are of course considering very low grade fuels—including various forms of refuse—so that strictures on the use of diffusion flames³ do not apply. Quite apart from the limitations imposed on maximum mean temperatures, maximum local temperatures are limited by the high diluent content of the stoichiometric mixtures which occur in the inter-diffusion zones.

Conclusions

Burners and combustion equipment generally have always been exceedingly simple, and given the availability of prime fuels and lack of concern about pollution, there is no reason why they should not have been. We have shown that such a minor escalation in complexity (in comparison with, for example, energy release by nuclear reactions) as the use of massive heat recirculation will greatly widen the limits to the release and utilisation of energy. It effectively abolishes limits of flammability, makes anything that is capable of exothermic reaction into a "fuel" and confines the limitations in energy conversion largely to those of the converter itself.

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Strained mixed-cluster model for glass structure

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Many features of glass behaviour can be explained by a new approach to glass structure based on two ideas. First, glass-forming melts generate 'clusters' of structurally non-related polymorphs which associate on cooling but cannot nucleate, and second, inter-cluster thermal strain can be relieved by subsequent 'plating-out' of modifier impurities.

GLASSES comprise a wide class of solids ranging from the familiar soda-lime-silica window glasses to relatively little known arsenide and phosphide based semiconducting materials¹⁻³. Perhaps the one feature all glasses have in common, and which defines an amorphous material as a glass, is a 'glass temperature', T_g . Above this temperature a glass behaves as a supercooled liquid, with a finite though possibly large viscosity; below T_g a glass is a solid, and, at least at low stresses, behaves elastically. T_g can be somewhat varied by cooling rate, and is associated with discontinuities in the temperature variation of many physical parameters, for example thermal expansion, specific heat and refractive index. Any convincing model of glass should be able to explain such behaviour.

Glasses usually show a number of other features, not all of which are readily understood on the basis of one or other of the generally accepted approaches to understanding glass: the network and microcrystalline models (for description see refs 4-7). Thus, there is evidence from X-ray diffraction studies that the short range order in glasses is similar to that in corresponding crystalline solids⁸⁻¹⁰, a conclusion echoed by Raman and backed up by infrared phonon studies (see, for example, refs 11 and 12). There is also evidence from small-angle X-ray scattering of 'micro-heterogeneities' in glass on the 20-Å scale¹³. Even more strikingly, changes in thermal expansion at temperatures corresponding to solid-state polymorphic transitions in related crystalline phases have been found in some oxide glasses, for example, in vanadium phosphate¹⁴ and even in pure silica (C. F. Drake, personal

communication). Such behaviour is of course easy to understand on the basis of a microcrystalline model of glass. What is less easy to understand in these terms, however, is the effect of adding 'impurities' on the viscosity, workability and recrystallisation characteristics of oxide glasses. This, of course, is the basis of the powerful semiempirical techniques developed in the glass industry for preparing 'good' glass: the complex compositions, with some constituents only at a concentration of about 1%, are not used just because only impure raw materials are available at an economic cost, but because they have a real effect, particularly on devitrification properties. A 'good' glass has a shallow viscosity-temperature characteristic and remains workable over a large range of temperature; it most certainly does not recrystallise. It is here that the network model seems particularly applicable, which may account for its almost universal adoption in the glass industry.

Could these two models be unified in some way to give a more general explanation of the properties of glass? The unconventional but simple approach outlined here does seem to suggest a means of achieving this. One point seems of particular relevance: a surprisingly large number of the systems which form glasses have as a major (sometimes the only) constituent a material that exists in two or more polymorphic forms which only differ slightly in free energy. Silica, as an obvious example, is found as quartz, cristobalite and tridymite (and in a number of other less wellknown forms too), but polymorphism is also found with BeF_2 , PbO , As_2O_3 , TiO_2 , selenium, sulphur and CdP_2 , to name several materials of interest in the context of glass formation. Could this polymorphism be a necessary condition for a material to be a glass former? Consider a melt of such a polymorphic material being slowly cooled. At some temperature, associations of the constituent atoms will grow into 'flicker-clusters', incipient nuclei of all possible polymorphs (any significant difference in free energy, of perhaps more than 5 kT, or about 0.1 eV at 1,000 K, would be expected to reduce greatly the contribution of the phase with higher free energy, although

bulk free energies may only be a rough guide to the situation with such small quasi-crystallites). The existence of flicker-clusters in liquids near their freezing point is well established; their formation for example, is the reason for the maximum density of water occurring at 4 °C (ref. 15).

The behaviour of such a system with two or more distinct types of flicker-cluster does not, however, seem to have been considered previously. I suggest that on progressively lowering the temperature such a 'mix' would not behave like the melt of a solid which has only one modification, and that it would be difficult for the clusters to grow to the critical size for nucleation. Instead bonds between clusters with different structure and at random mutual orientation would, initially, continuously form and break, the tendency for their forming increasing as temperature was reduced. The resulting 'mixed-cluster associations' could, presumably, greatly exceed the critical size for nucleation for a single phase cluster without passing the surface energy determined maximum in free energy beyond which runaway crystallisation would normally occur. (Such a situation would give a melt in which viscosity would be expected to increase markedly as temperature fell.) Given this, a temperature would be reached at which a permanent, continuous, bonded network would be set up between mixed clusters, leaving much of the boundaries of the clusters non-bonded and with the material between them still 'liquid-like', even though the system as a whole would have now 'locked up solid', a rigid rather than a viscous material having come into being. Presumably this situation corresponds to some kind of percolation theory limit. It would of course also correspond to T_g . In addition, it is clear from this picture that slow cooling would give rise to a better accommodated continuous network of this kind than fast cooling, leading to just the behaviour found experimentally with T_g in terms of density, refractive index and so on.

An objection to this could be that if two types of cluster were present, A and B, then A-A and B-B contacts could occur as readily as the A-B type discussed above and would tend to lead to nucleation. The important point is, however, that the occurrence of A-B random bonding would hinder and eventually suppress subsequent A-A or B-B epitaxial contacts, which are what are required to reduce surface free energy.

Consider what happens on still further cooling. The liquid and presumably relatively mobile material would remain within the rigid mixed cluster framework gradually crystallising out, but with progressively more distorted bonding arrangement as cluster-cluster boundaries and voids (due largely to thermal contraction) are formed. There would, however, be a crucial further effect operating at the same time. Since the two (or more) cluster-phases would have different crystal structure symmetries, and would be bonded together with more or less random orientation, there must be thermal contraction mismatch between them. This would give rise to progressively increasing strain on cooling which would tend to increase the free energy of the system, making it increasingly unstable with respect to a more normally crystalline variant. (This term then might be particularly important in driving towards devitrification of a glass even above T_g .) It is here that I would suggest that the liquid material bonding out on to mixed clusters is important. Particularly if impurity atoms of correct size were present ('modifiers'), these would grow on to the cluster surfaces or associate at cluster-cluster boundaries in such a way as to reduce the strain contribution to the free energy and also, presumably, the entropy of the system. This, then, qualitatively explains the importance of small additions of specific impurities in stabilising the glassy state. This mechanism, incidentally, is in marked contrast with one put forward by Demishev¹⁶ who postulated microstresses between "an ordered phase" and "disordered material" due to differences in the thermal expansion coefficients of the two kinds of material, which coincide at T_g .

This phenomenological picture of how a glass forms suggests some interesting questions and predictions:

(1) The existence of liquid material with high atomic mobility

below T_g (albeit on an extremely small scale) should be amenable to direct verification by means of motional narrowing of nuclear magnetic resonance if a suitable isotope were available (¹¹B in borate glasses?), although presumably a sensitive apparatus might be needed since only a small proportion of the material would be expected to be involved.

(2) The mixed-cluster model also suggests that immediately below T_g a glass is very like a liquid-filled zeolite, the liquid being held in a system of small interconnecting channels, and with a very large solid surface in contact with it. Interesting effects of ion exchange and diffusional transport should be possible. This view is consistent with the known and highly important "gettering action" of glass films on the surface of silicon semiconductor devices.

(3) Perhaps on the basis of intuition more than argument, it may be possible in a particularly favourable case of glass-forming material and strain-relieving impurities to obtain a system with a lower free energy than any corresponding combination of macrocrystalline phases, that is a glass might be more stable than crystalline material—an almost heretical suggestion but worth further investigation.

(4) If more than the optimum amount of strain-relieving impurity were present in a glass system, the glass would become less stable, simply because there would be a tendency for a new phase to form in the now relatively thick layer of "extra-cluster material" which compositionally would be different from the clusters. If, however, this itself has suitable polymorphs a new mixed cluster system could arise, and still further addition of impurity could restabilise the system. This may offer a generalised explanation for the not uncommon occurrence of compositional systems which show broad glass-making fields, with varying glass stability, for example in the alkali silicates. The important new point here is that in such systems the glass former can be an intermediate compound rather than having to be an end member.

(5) If, as seems possible, the mechanism of strain relief by "plating out" from residual liquid could be a selective process operating not far from equilibrium, this may lead to an explanation of at least the low temperature behaviour summarised under the heading of "the mixed alkali effect" in silicate glasses¹⁷. The simultaneous presence in the liquid of two different-sized alkali atoms would then be expected to lead to a better packed and strain-relieved structure than either could give separately (for example, spheres of two different sizes can pack to fill space more efficiently than a single size of sphere) and thus a denser, more strongly bonded, higher refractive index glass. Such a structure would also account for the observed reduction in alkali diffusion rate in mixed alkali glasses.

(6) The case of vitreous silica is of particular interest. Detailed radial distribution function analyses by Konnerth and Karle^{8,9} showed the presence of tridymite-like quasi-crystallites perhaps 20 Å across and gave some indication that cristobalite-like material may also be present. As already noted, work in these laboratories by Drake (C. F. Drake, personal communication), using a sensitive dilatometer of his own design, has indicated changes in the slope of the thermal expansion curve of silica (from a variety of sources) which have been persuasively identified as associated with α - β transitions in quasi-crystallites of cristobalite and quartz. There is evidence, therefore, that vitreous silica contains at least three types of cluster. Now Cohen and Roy^{18,19} found that the density of vitreous silica is affected irreversibly by the application of pressures of more than 2×10^9 Pa. This can be interpreted on the present model as due to quasi-crystallites of the least stable phase, tridymite, going over to a more dense coesite-like structure. (The quartz-coesite transition is near 3×10^9 Pa (ref. 20) for bulk material; for tridymite a somewhat lower transition pressure would be expected.) If so, a Konnerth and Karle type RDF analysis of such densified material should be very different to that which was found for normal vitreous silica. At a sufficiently high pressure of course all quasi-crystallites would become transformed to rutile-like stishovite²¹ and recrystallise-

tion should occur immediately since no "two-or-more-cluster-phase-system" would be in question. Cohen and Roy^{18,19} in fact mention just such behaviour for GeO₂ glass, which would be expected to transform into a rutile-like structure at a lower pressure than for the case of SiO₂. The role of shear in such transformations²² does, however, require further investigation.

(7) It should be possible to distinguish between amorphous solids and those which might, potentially at least, be stabilised as glasses. Amorphous silicon and germanium, for example, can probably not be stabilised, since there seem to be no alternative crystal structures within a reasonable energy range, judging from the very high pressures and compressions required to bring about any phase transformation²³. It might, however, be argued, if the two-polymorph idea is correct, that quenching a germanium melt at a pressure sufficiently high for the freezing point to lie near the phase boundary between (metallic) tetragonal and (semiconducting) diamond type phases could perhaps give rise to a glass. More interesting possibly, is the case of carbon, where at 1 atm pressure ($\sim 10^6$ Pa) graphite and diamond phases differ in free energy by about 500 cal mol⁻¹ (~ 0.03 eV). This suggests that it may be possible to quench liquid carbon to give a glass. Could this be stabilised by transition metals such as iron? Is there any evidence of high viscosity due to graphite-diamond quasi-crystallite cluster formation in the "solvent catalyst" melts used for diamond synthesis, whose mode of action is still not too well explained?²⁴

It also seems likely that where two polymorphs are closely related in structure (for example wurtzite and zinc blende) and have not only the same coordination but differ solely in stacking (so that epitaxial relationship is possible), then the suppression of nucleation described above would be much less likely to occur. (In this context the findings that cristobalite²⁶ and tridymite (J. H. Konnert and J. Karle, personal communication) do not have the simple structures previously assumed to be zinc blende and wurtzite analogues are highly relevant.) For this reason, then, amorphous III-V and II-VI compounds, much like silicon and germanium, should not form glasses. Some reservation may be needed here in that glasses have been made from the related ternary chalcopyrite compounds CdGeAs₂ and CdGeP₂ (refs 1-3). In these two cases the structures of the melts seem to be very different to that found for the simpler diamond-like solids: they have a much lower coordination number, and the binary compounds CdAs₂ and CdP₂ seem to be the glass forming materials.

(8) There is an interesting parallel between the formation of mixed clusters and the behaviour of an eutectic mixture on cooling. At the eutectic temperature two phases crystallise simultaneously but, usually, on a scale which is orders of magnitude larger than has been discussed so far. If it were possible, however, for example by fast cooling, to induce a mixed cluster type structure, the result should be a glass. This may account for the formation of metallic glasses such as Pd₈₀Au₁₁Si₉ by splat cooling²⁵. Such materials tend to recrystallise near T_g as would be expected from this model.

(9) A particularly interesting possibility is that cluster-cluster interfaces (with their attendant strains and voids) at the surface may be associated with the generation of Griffith cracks, which limit the tensile strength of all glasses. Rindone²⁷ notes that "... microheterogeneities of different composition will have different chemical reactivities, especially with water. For example, a potassium-rich region would react with water much more rapidly than would a sodium-rich region. This could lower the strength considerably if there are local tensile stresses around the microheterogeneity due to thermal expansion differences ..."

The strained mixed-cluster model, therefore, offers explanations for a wide range of properties found in glasses and can also be used to predict interesting new possibilities. It is, however, only a descriptive approach and obviously requires detailed development before it could be used in anything but the crudest manner. It also may not be applicable to glasses formed from two- or one-dimensional crystal structures, such as long chain polymers, or, even, As₂S₃. This last material in fact seems to be known only in one crystal structure (even though there is a mention of a red β -As₂S₃ in the old literature²⁶) and so, formally, may be an exception to the rule proposed above.

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Magnetic vibrational optical activity in the resonance Raman spectrum of ferrocytochrome c

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The first observations of magnetic Raman optical activity are reported. Using a field of about 0.7 T, Raman circular intensity differentials up to $\pm 4 \times 10^{-3}$ are induced in resonance Raman bands of ferrocytochrome c.

OPTICAL activity associated with molecular vibrations can now be studied through a difference in the intensity of Raman scattering in right and left circularly polarised incident light¹⁻⁴.

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Effects found so far in naturally optically active molecules indicate that the Raman circular intensity differential (CID) is a powerful probe of configuration and conformation. All molecules are rendered optically active by static magnetic fields, and should show Raman CIDs if the magnetic field is parallel to the incident laser beam⁶. Magnetic Raman optical activity has not been observed so far at transparent frequencies, but is reported here in the resonance Raman spectrum of ferrocyclochrome *c*, a haem protein, using a modest magnetic field.

There are many problems in the application of conventional vibrational spectroscopy to biological molecules. Infrared radiation is absorbed strongly by water, the ubiquitous biological material, and the complexity of biological molecules can lead to thousands of normal modes of vibration. Raman spectroscopy does not suffer from the first limitation as water does not absorb visible and near ultraviolet wavelengths and scatters them only weakly. The second limitation can be overcome through the resonance Raman technique in which the laser frequency falls within an electronic absorption band, usually localised at a site of biological function such as a haem group, resulting in a tremendous enhancement of the Raman intensities of those few vibrations coupled to the electronic transition⁶.

It would clearly be of interest if CIDs could be observed in the resonance Raman spectra of biological molecules as vibrational optical activity, both natural and magnetic, is expected to be sensitive to the delicate configurational and conformational features that determine biological function. Attempts to observe natural optical activity in the resonance Raman spectra of haem proteins and visual pigments on retinal membranes have not been successful so far, possibly because electron conjugation tends to minimise the chirality by holding the chromophores nearly planar. But as porphyrin rings are very susceptible to magnetic perturbations and show large magnetic, optical rotatory dispersion and magnetic circular dichroism^{7,8}, haem proteins are extremely suitable for the observation of magnetic resonance Raman optical activity. Furthermore, the resonance Raman phenomenon enables the contributions from those electronic transitions most susceptible to magnetic perturbations to be isolated, whereas in transparent regions all electronic transitions contribute to Raman scattering.

The quantity that is measured is a dimensionless CID in the light scattered at 90°:

$$\Delta_\alpha = (I_\alpha^R - I_\alpha^L) / (I_\alpha^R + I_\alpha^L) \quad (1)$$

where I_α^R and I_α^L are the scattered intensities with α -polarisation in, respectively right and left circularly polarised incident light. If z is the direction of the incident beam, I_z and I_x are linearly polarised parallel and perpendicular to the scattering plane, yz (Fig. 1). Δ_z and Δ_x are referred to, respectively, as the depolarised and polarised CIDs.

Magnetic Rayleigh and Raman optical activity originate in interference between the scattered electromagnetic waves whose

Fig. 1 The geometry for polarisation experiments in Raman scattering at 90°. z , Direction of the incident beam; y , direction of the detected beam; R , right circular polarisation; L , left circular polarisation.

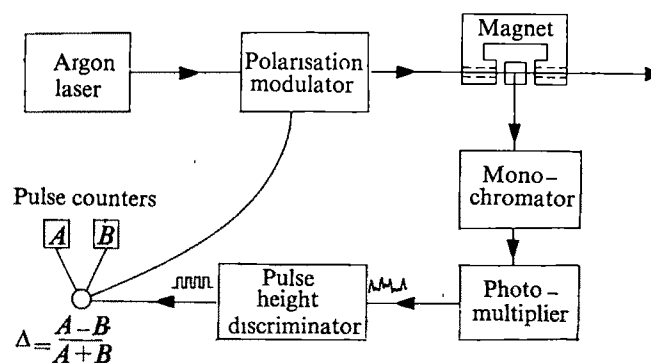
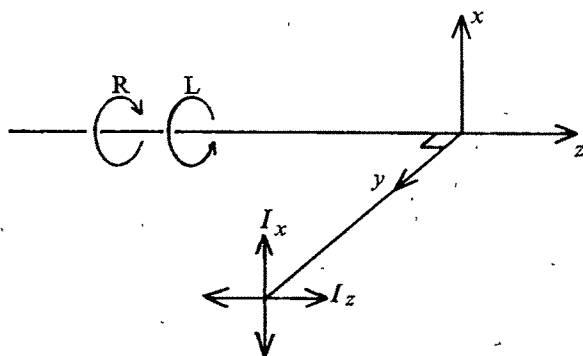


Fig. 2 The experimental arrangement for measuring magnetic Raman CID. A beam from an argon-ion laser is brought to a broad focus in the sample, which is held in a quartz fluorescence cell with a path length of 1 cm. The sample cell is situated between the poles of a permanent magnet with holes bored through the pole pieces so that the magnetic field is parallel to the laser beam. The light scattered at 90° is processed by a triple monochromator and the Raman components detected by a photomultiplier which produces measurable voltage pulses from individual photon strikes. These pass through a discriminator which gives equal weight to all pulses which exceed a certain voltage threshold; this enables much of the noise from the photomultiplier to be eliminated, and gives the scattered intensity as a 'photon' count rate. The polarisation of the incident beam is modulated directly between right and left circular at 9 Hz using a potassium-dihydrogen phosphate crystal driven by a square wave alternating voltage synchronised with two matched pulse counters. While the modulator performs the first half of its cycle and produces right circular polarisation, pulses are channelled into counter *A*; while the modulator performs the second half of its cycle and produces left circular polarisation, pulses are channelled into counter *B*. After accumulating a statistically significant number of counts, the sum of the two counters $A+B = I^R + I^L$ and the difference $A-B = I^R - I^L$ are read. The statistical significance of N counts is determined by the standard deviation \sqrt{N} . Thus, on 10^8 counts, the 'statistical noise' is $\sqrt{N}/N = 10^{-4}$, which is an order of magnitude smaller than the larger CIDs expected. Only the depolarised CIDs are sampled (see refs 2 and 4).

amplitudes are proportional to the usual electric dipole-electric dipole polarisability tensor and the same tensor perturbed to first order in the component of the magnetic field along the direction of the incident beam. The basic theory of magnetic Rayleigh and Raman CID was worked out by Barron and Buckingham⁶, although the final expressions apply only to Rayleigh and Raman scattering at transparent frequencies and to Rayleigh scattering at absorbing frequencies. That is because at transparent frequencies, to a good approximation, the real part of the transition polarisability is purely symmetric and the imaginary part is purely antisymmetric; but at resonance this approximation breaks down completely and, in general, the real transition polarisability contains an antisymmetric part and the imaginary transition polarisability contains a symmetric part.

The arrangement for measuring magnetic Raman CID is illustrated in Fig. 2.

One problem anticipated with resonance Raman CID measurements is a bias caused by the circular dichroism of the incident beam. But none was detected to within $\pm 1 \times 10^{-4}$, probably because the magnetic circular dichroism of ferrocyclochrome *c* at 5,145 Å is very small⁹. In general, this problem could be overcome by tuning the laser frequency to coincide with the one or more nodes that often occur within a magnetic circular dichroism band.

The depolarised magnetic resonance Raman CID spectrum of ferrocyclochrome *c* is shown in Fig. 3. There is good reflection symmetry on reversing the magnetic field direction, and no significant CIDs are found when the field is removed. Magnetic CIDs occur in most of the strong resonance-enhanced Raman bands between 1,100 and 1,600 cm^{-1} . No significant effects are found in the weaker bands in other parts of the spectrum.

These magnetic Raman CIDs involve vibrations of symmetry species B_1 , B_2 and A_2 , assuming an effective symmetry of C_{4v} .

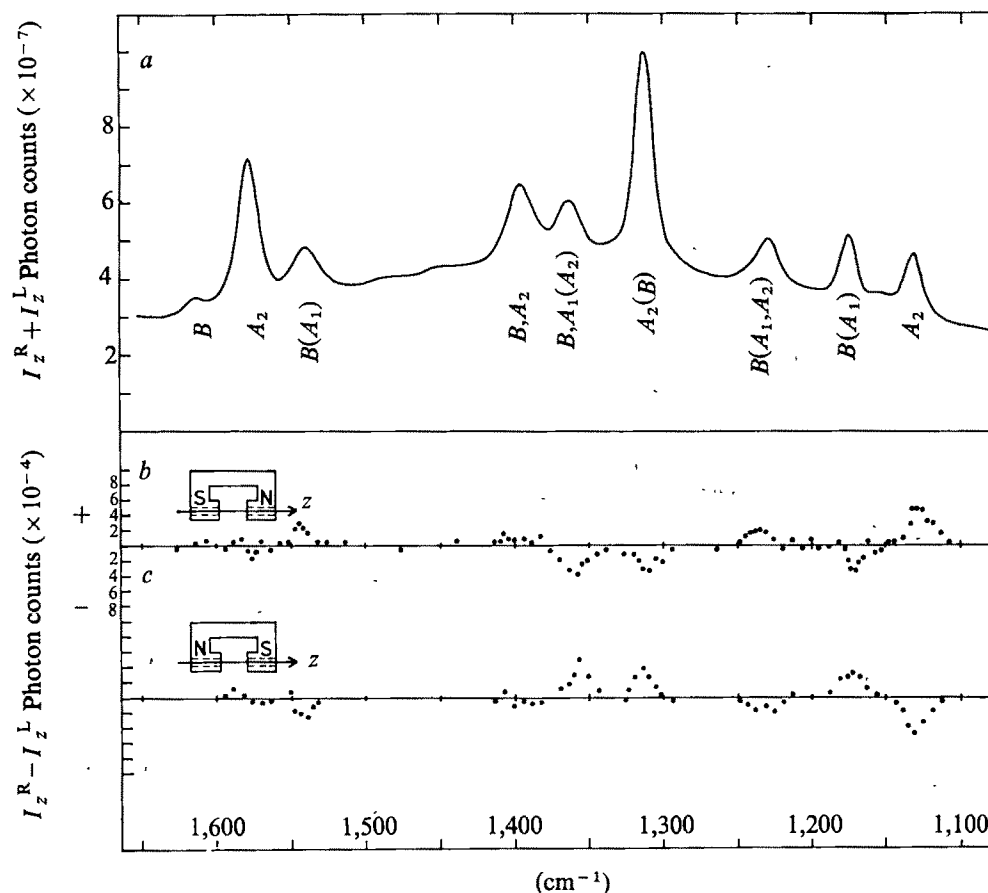


Fig. 3 The depolarised magnetic circular intensity sum (a) and difference (b and c) spectra of ferrocytochrome c. The magnetic field directions are opposite in b and c. Experimental conditions: 1×10^{-4} M aqueous solution of cytochrome c with excess sodium dithionite; magnetic field strength about 0.7 T; laser wave-

length, 5,145 Å; laser power, 500 mW; slit width, 13 cm⁻¹. The $I_z^R - I_z^L$ spectra were recorded manually at fixed points with a counting period of about 8 min. To estimate the dimensionless CIDs, the background should first be subtracted from the $I_z^R + I_z^L$ spectrum.

for the porphyrin ring in ferrocytochrome c, and the symmetry assignments are indicated in the top spectrum. In brackets are shown the minor contributors that arise because the planar tetragonal symmetry of the basic porphyrin ring, which provides a useful first approximation to the classification of the spectroscopic properties, is actually distorted by thioether side groups which link the haem to the protein¹¹.

The resonance Raman scattering process involves transitions from the zero vibrational level of the porphyrin ground electronic state to higher vibrational levels of the first π^* excited electronic state (${}^1E_u \leftarrow {}^1A_{1g}$)¹². Since the ground electronic state is non-degenerate there is no ground state magnetic moment, so the observed magnetic Raman CIDs should be independent of temperature. And as the excited electronic state is doubly degenerate the parts of the magnetically-perturbed transition polarisability that correspond to generalised forms of the Faraday A term of magnetic circular dichroism¹³ should dominate.

A detailed analysis of the magnetic Raman CID spectrum would be premature since the theory is not sufficiently developed and the spectrum is poorly resolved. It is, however, worth noting that the largest magnetic CID ($|\Delta_z| \sim 4 \times 10^{-3}$) is found in the Raman band at about 1,132 cm⁻¹, which originates in a single vibrational mode of A_2 symmetry. Since the corresponding transition polarisability is completely antisymmetric, the band shows the striking phenomenon of inverse polarisation¹⁴, which has so far only been found in resonance Raman bands and is critically dependent on the laser wavelength¹⁵. The other A_2 modes show only weak or negligible magnetic CIDs, and when interpreted these relative CID intensities and their variation with laser wavelength may lead to a better understanding of the

molecular mechanism of antisymmetric resonance Raman scattering, which is still obscure.

The interpretation of the magnetic CID spectrum will be facilitated when examples are found in other molecules, particularly free porphyrins and haemoglobins, in which the chromophore symmetry is better defined so that there is less mixing of the vibrational modes. Unfortunately, it has not yet been possible to obtain results from free porphyrins or haemoglobins since they do not survive long enough in the intense laser beam to allow significant CID measurements to be taken. But with more elaborate sampling arrangements and a stronger magnetic field (an order of magnitude increase is available with superconducting magnets), magnetic Raman optical activity should be accessible in many other samples.

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letters to nature

No indication of excess MeV gamma radiation from the Crab Nebula

DURING the past two years results from two different balloon experiments^{1,2} indicate that the γ -ray emission from the Crab Nebula between 1 and 10 MeV is considerably above the straight line interpolation from lower and higher photon energies. The excess above the power law has been claimed to be factor of 7 between 1 and 3 MeV and of a factor 30 between 3 and 10 MeV. The fact that both experiments yielded nearly identical results gives support to the reality of the excess component. The power in this excess is nearly as large as the total integrated synchrotron power of 2×10^{-7} erg cm⁻² s⁻¹ of the Crab Nebula. It has been speculated that this powerful, narrow-band component may be produced in the pulsar magnetosphere, possibly near the surface of the neutron star².

Results of a balloon flight with our double Compton telescope^{3,4} now set an upper limit to the soft γ -ray flux between 1.5 and 10 MeV from the Crab Nebula which is in severe contradiction to the flux values published previously^{1,2} and rules out the existence of an excess—at least of this magnitude.

The telescope consists of two large plastic scintillator blocks, 1.20 m vertically apart. Both detectors are surrounded by an anticoincidence shield. A γ ray is identified by a first Compton collision in the upper detector and a second in the lower detector—this sequence being confirmed by a time-of-flight measurement. Energy and direction of the γ rays are determined by pulse height measurements in both detectors. The telescope has an acceptance cone of 26° half width at half maximum (HWHM) between 1.5 and 2 MeV, 23° between 2 and 3 MeV, 18° between 3 and 5 MeV and 14° between 5 and 10 MeV. The energy resolution is 40% FWHM at 2.75 MeV. The effective geometrical area for vertical incidence is zero below

1 MeV, increases to 1.6 cm² at 1.5–2 MeV, has a maximum of 2.6 cm² at 3–5 MeV and drops to 2.2 cm² at 5–10 MeV.

The transit of the Crab Nebula was 162 min after the balloon was launched. Because of the large acceptance cone of the telescope the detection probability of γ rays from the Crab drops to half its maximum value ~ 100 min after the transition and is zero ~ 50 min later. In Fig. 1 the total 1.5–10-MeV counting rate is plotted as a function of time after launch. Only those events are taken into account, which satisfy the so called $\bar{p} < 30^\circ$ restriction³ and which fall into a certain time-of-flight interval. No statistically significant excess of the counting rate is present over the full observation period. This is also true, if the total energy range is subdivided into four smaller intervals. Although events have also been triggered with energies below 1.5 MeV, these have not been taken into account to avoid uncertainties in the detection efficiency, which are caused at these energies by its strong dependence on the accurate setting of the trigger levels.

For each 20-min interval in Fig. 1 an upper flux limit of the Crab Nebula at the 95% confidence level was determined according to

$$r_{2\sigma} = \int_{1.5}^{10} S(E_\gamma) A \varepsilon(E_\gamma, \theta) f_i \exp \left[-\frac{2.5 \text{ g cm}^{-2}}{\lambda(E_\gamma) \cos \theta} \right] dE_\gamma \quad (1)$$

where $r_{2\sigma}$ is the 1.5–10 MeV 2σ counting rate uncertainty in Fig. 1; A is the detector area; $\varepsilon(E_\gamma, \theta)$ is the energy and zenith angle dependent detection efficiency; f_i is the fraction of events, for which the time of flight requirement is fulfilled; $\lambda(E_\gamma)$ is the

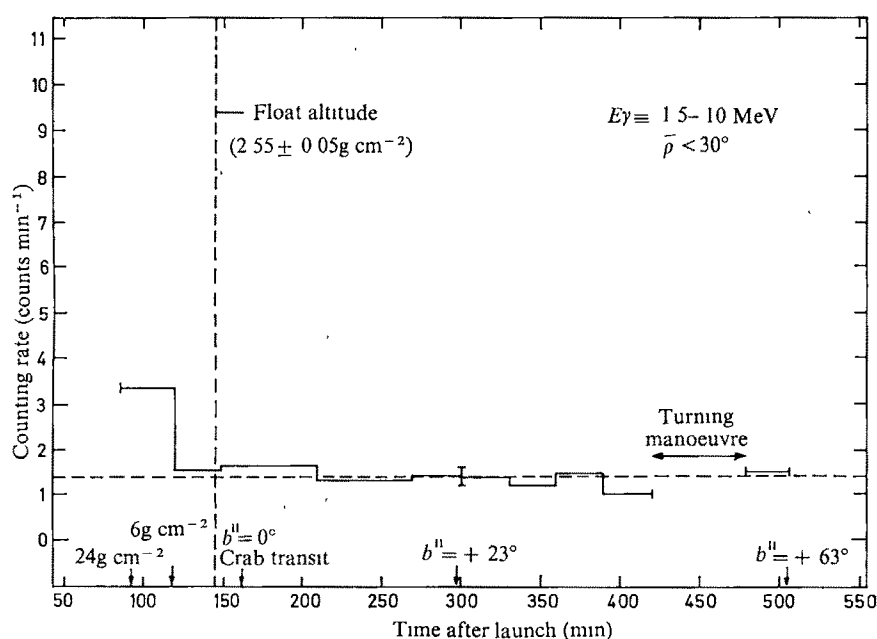


Fig. 1 1.5–10-MeV counting rate of the double Compton telescope as a function of time after launch. Only those events are taken into account for which a certain time of flight requirement and the so-called $\bar{p} < 30^\circ$ restriction³ is fulfilled. The balloon was launched from Palestine, Texas on July 11, 1974 at 0905 LT. The balloon reached its float altitude of 2.5 g cm⁻² within 145 min. During the first 4.6 h at float the telescope axis was adjusted to the zenith. Within the next 58 min the axis was rotated from 0 to 360° zenith angle to measure the zenith angle distribution of atmospheric rays. During the last 28 min at float altitude the axis was again adjusted to the zenith. Results from this flight on the diffuse cosmic γ -ray component and on the angular distribution of atmospheric γ -rays have already been published^{5,6}.

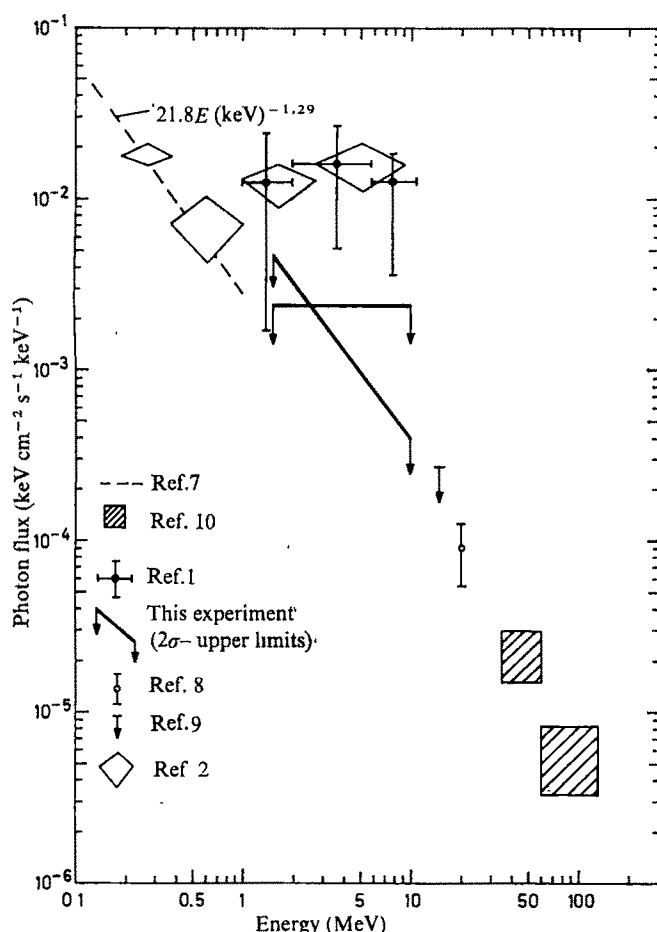


Fig. 2 Energy spectrum of the Crab Nebula. Our upper limits at the 2σ confidence level were derived from the assumption of a power law spectrum between 1.5 and 10 MeV of the form $\sim E^0$ or $\sim E^{-1.3}$.

mean free path of γ rays in the atmosphere; θ is the time-dependent zenith angle of the Crab Nebula; and $S(E_\gamma)dE_\gamma$ is the differential photon number spectrum of the Crab Nebula. For the Crab spectrum a power law with two different exponents has been considered:

$$S(E_\gamma)dE_\gamma = a_1 E_\gamma^{-\alpha_1} dE_\gamma \quad \text{photons cm}^{-2} \text{ s}^{-1} \text{ MeV}^{-1} \quad (2)$$

with $\alpha_1 = 1$ and $\alpha_2 = 2.3$. The first case describes the spectral form as determined by Baker *et al.* and Gruber, the second one is identical with the interpolation from lower and higher photon energies. For each 20-min interval the value for the proportional factor a_1 or a_2 was then determined applying equation (1). The most significant upper limit for the flux of the Crab Nebula is obtained if the limits of each interval from observation periods between 20 min before and 80 min after the transit are averaged quadratically. Then $a_1 = 2.41 \times 10^{-3}$ and $a_2 = 8.24 \times 10^{-3}$. These limits are shown in Fig. 2, where the energy spectrum of the Crab Nebula is shown from X-ray energies to be about 100 MeV.

The upper limit determined from our flight is at least a factor of 5 below the flux values quoted by Baker *et al.*¹ and Gruber² and is only a factor of 3 above the straight line interpolation from lower and higher energies.

If the flux values as determined by Baker *et al.* and Gruber were correct, the total counting rate of our telescope would have been a factor of 3 higher during the transit compared with the counting rate in the second half of our flight. In comparison, the flux value of Gruber was determined from only a 5% increase of the total counting rate; the increase shown by Baker

et al. was even less than 1%. Variations of this order are easily obtained by temperature shifts, electronic instabilities or geomagnetic effects. During our flight at float the temperature inside the gondola did not change by more than 3.5 K. Even if as a consequence the gain had shifted by 20%, which is a very extreme assumption, the counting rate shown in Fig. 1 would have changed at most by 40%, which is just as large as the 2.5σ uncertainty. The geomagnetic latitude continuously decreased from 42°N to 39°N during the flight. Therefore, the flux of the Crab Nebula can at most be overestimated, but cannot be underestimated. We conclude, therefore, that the excess of γ radiation from the Crab Nebula as reported previously^{1,2} does not exist. From our upper limit one could expect that the flux accepts the value of the straight line interpolation.

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Ultraviolet photoabsorption by halocarbons 11 and 12 from electron impact measurements

DEPLETION of the stratospheric ozone layer by chlorine atoms released through solar photolysis of the chlorofluoromethanes is a matter of widespread concern¹. Predictions of the magnitude and time development of this effect depend on large scale models of atmospheric transport and photochemical kinetics which require reliable data on the primary reaction processes involved. One important input is the ultraviolet absorption spectrum of halocarbons 11 and 12, that is, CFCl_3 and CF_2Cl_2 , respectively. Published optical determinations² of the absorption spectra for these molecules between 200 and 120 nm (that is, 6.2 and 10.33 eV) have been called into question, however, by the measurements of Rowland and Molina³.

We have obtained oscillator strength distributions for a number of molecules^{4,5} from high quality electron energy-loss spectra. Previous studies have shown excellent agreement (better than $\pm 15\%$) between electron impact and optical values below 15 eV, that is, for wavelengths longer than 80 nm.

Electron energy-loss spectra for CFCl_3 and CF_2Cl_2 were obtained digitally with the NBS model AN-1 electron impact spectrometer⁵ for 100 eV electrons scattered within 20 mrad of the incident direction. Halocarbon gases were obtained commercially and used without further purification. Subsequent analysis by gas chromatography confirmed a purity greater than 99.8% with the only identifiable impurity being 0.1% CF_2ClH in CF_2Cl_2 .

Relative oscillator strength distributions for CFCl_3 and CF_2Cl_2 were derived by a procedure⁶ that corrects for the finite

angular acceptance of the apparatus. For CF_2Cl_2 (halocarbon 12) the relative distribution was then normalised at an energy loss of 12.22 eV to a value $dI/dE = 0.732 \text{ eV}^{-1}$ obtained from an unpublished photoabsorption cross section of $80.3 \pm 2.4 \times 10^{-18} \text{ cm}^2$ measured by J. C. Person and coworkers at 101.4 nm. Their absolute accuracy of $\pm 3\%$ is much better than that quoted by Gilbert *et al.*⁶. Our values are within $\pm 5\%$ of the measurements of Person *et al.* between 12 and 15 eV but are nearly a factor of 1.4 higher than those of Gilbert *et al.*⁶ in this region. Between 10.4 and 11.4 eV, however, our values agree well with those of Gilbert *et al.*⁶. In fact, our disagreement with their optical data occurs mainly in the spectral region where they used a windowless absorption cell. This leads us to suspect some undetermined error in their measurements associated with the absence of windows.

For CFCl_3 (halocarbon 11) only the data of Gilbert *et al.*⁶ are available for normalisation. Since we obtain good agreement with their values between 10.4 and 11.4 eV for halocarbon 12, we have normalised our halocarbon 11 data at 10.76 eV to $dI/dE = 0.906 \text{ eV}^{-1}$. This value corresponds to their molar extinction coefficient⁶ of $26,000 \text{ l mol}^{-1} \text{ cm}^{-1}$ measured at 115.2 nm. With this normalisation we found agreement with their values below 11.4 eV, but again in the windowless region our values are nearly twice as large. A more detailed analysis of the vacuum ultraviolet region for a large set of halocarbons is in preparation.

Our energy absorption results relevant to the ozone depletion problem are shown in Figs 1 and 2. A portion of our electron-impact data are compared with the optical measurements of

Fig. 1a, Comparison of oscillator strengths for halocarbon 11 (CFCl_3) in the ultraviolet absorption region: —, present electron impact results; \times , optical values from ref. 2; Δ , optical values from ref. 2 as quoted in ref. 3; \bullet , optical values from ref. 3. b, Comparison of oscillator strengths for halocarbon 12 (CF_2Cl_2) in the ultraviolet absorption region (identifications as in a).

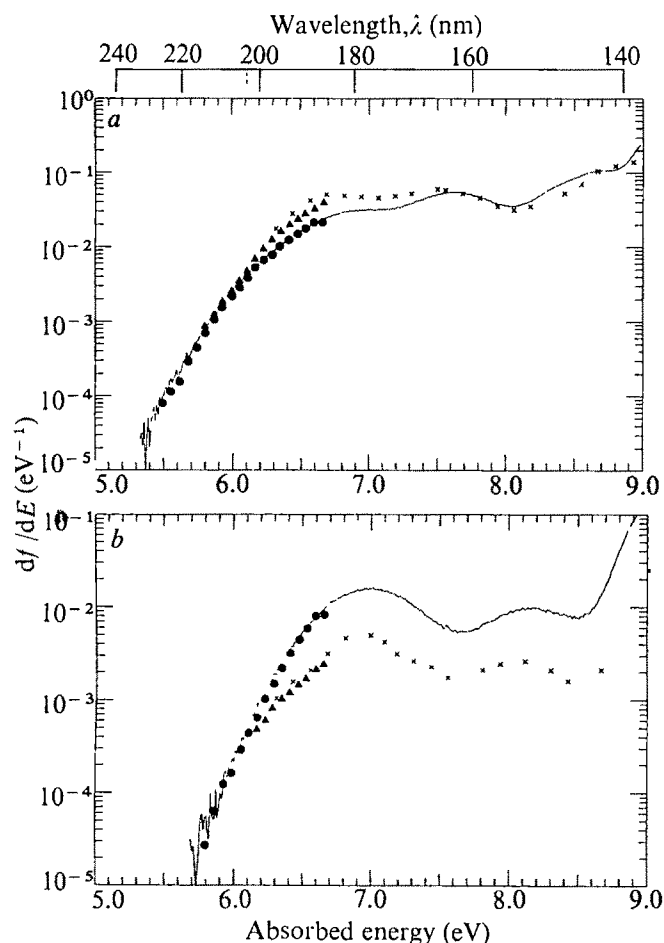


Table 1 Apparent photoabsorption cross sections for CFCl_3 and CF_2Cl_2 derived from electron energy-loss measurements

λ (nm)	Apparent photoabsorption cross section* (cm^2) CFCl_3	CF_2Cl_2
225	1.11 (−20)	—
221	2.20 (−20)	—
218	4.55 (−20)	1.97 (−21)
214	9.77 (−20)	4.50 (−21)
210	1.64 (−19)	1.08 (−20)
207	2.90 (−19)	2.63 (−20)
203	4.77 (−19)	5.16 (−20)
200	6.82 (−19)	1.02 (−19)
197	9.77 (−19)	2.10 (−19)
194	1.35 (−18)	3.44 (−19)
191	1.78 (−18)	5.65 (−19)
188	2.29 (−18)	8.42 (−19)
185	2.77 (−18)	1.15 (−18)
182	3.23 (−18)	1.49 (−18)
177	3.49 (−18)	1.76 (−18)
172	3.68 (−18)	1.39 (−18)
168	4.75 (−18)	8.91 (−19)
163	5.88 (−18)	5.98 (−19)
159	5.15 (−18)	6.87 (−19)
155	3.89 (−18)	9.46 (−19)
151	4.76 (−18)	1.07 (−18)
148	7.74 (−18)	9.45 (−19)

*The number in parentheses is the exponent of the power of ten to be multiplied by the value of the cross section tabulated

Doucet *et al.*² and the work of Rowland and Molina³. The oscillator-strength distributions we obtain for halocarbons 11 and 12 include energy absorption in the so-called "window" region of the solar radiation flux³. These data extend from the Hartley band of O_3 (maximum at 4.9 eV or 255 nm) to the Schumann–Runge continuum of O_2 (maximum at 8.5 eV or 146 nm). Our data confirm the measurements of Rowland and Molina³ for both halocarbons and disagree by as much as a factor of two for halocarbon 11 and a factor of four for halocarbon 12 with earlier measurements². Further, our results differ from those of Doucet *et al.*² over a significant portion of the absorption spectrum at higher energies for both halocarbons.

One can calculate apparent photoabsorption cross sections from the present results (Fig. 1) by multiplying by a conversion factor of $1.0975 \times 10^{-16} \text{ cm}^2 \text{ eV}$. Justification of our procedure for obtaining apparent photoabsorption cross sections from electron impact data depends on the theory of limiting oscillator strengths⁷ and is subject to the same qualifications we have discussed previously⁶. Since these data are relevant to atmospheric model calculations assessing the impact of continued halocarbon releases, the apparent photoabsorption cross sections obtained from our measurements are given in Table 1.

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Aromatic units in coal

THE organic matter in coal consists largely of a macromolecular material of complex and variable composition. When coal is pyrolysed, a large fraction is converted to char, and a number of complex aromatic compounds are formed. Because of the

extensive chemical change which takes place during this process, however, it is not clear what relationship the products have to the original structure of coal. Many workers have used oxidation procedures to degrade coal to simpler species which are more readily interpreted in terms of coal structure. Oxidation products, however, were in general studied by limited classical organic chemistry methods for separation and identification of products^{1,2}. To date the only aromatic acids definitely identified have been the carboxylic acids of benzene^{3,4}. Commonly used oxidants have been HNO_3 , $\text{HNO}_3\text{-K}_2\text{Cr}_2\text{O}_7$, KMnO_4 , O_2 and $\text{H}_2\text{O}_2\text{-O}_3$ in conditions for drastic degradation of aromatic rings. On the basis of results of oxidation of coal with a selective oxidant, NaOCl , Chakrabarty and Berkowitz have suggested⁵ that coal has a largely non-aromatic "tricycloalkane or polyamantane" structure. They pointed out that no evidence for aromatic compounds other than benzene derivatives was found in their oxidation product. Our experimental data do not support

Table 1 Aromatic acids found in the aqueous $\text{Na}_2\text{Cr}_2\text{O}_7$ oxidation of coal, and identified as methyl esters

Nucleus	Number of $-\text{COOCH}_3$	Elemental composition	Precise mass ($\text{M}-\text{OCH}_3$) ⁺		Relative* abundance ($\pm 15\text{-}20\%$)
			Observed	Deviation $\times 10^3$	
Benzene	2	$\text{C}_9\text{H}_7\text{O}_3$	163.0400	0.6	38
	3	$\text{C}_{11}\text{H}_9\text{O}_5$	221.0463	1.4	100
	4	$\text{C}_{13}\text{H}_{11}\text{O}_7$	279.0516	1.2	64
	5	$\text{C}_{15}\text{H}_{13}\text{O}_9$	337.0559	0.1	11
	1	$\text{C}_8\text{H}_7\text{O}_2$	135.0452	0.6	17
OCH_3 -benzene†	2	$\text{C}_{10}\text{H}_9\text{O}_4$	193.0518	1.8	17
Benzophenone	1	$\text{C}_{14}\text{H}_9\text{O}_2$	209.0582	-1.9	8
	2	$\text{C}_{16}\text{H}_{11}\text{O}_4$	267.0677	2.0	9
Naphthalene	1	$\text{C}_{11}\text{H}_7\text{O}$	155.0488	-0.8	1
	2	$\text{C}_{13}\text{H}_9\text{O}_3$	213.0547	-0.4	9
	3	$\text{C}_{15}\text{H}_{11}\text{O}_5$	271.0610	0.6	27
	4	$\text{C}_{17}\text{H}_{13}\text{O}_7$	329.0654	-0.6	8
CH_3 -naphthalene	1	$\text{C}_{12}\text{H}_9\text{O}$	169.0618	-3.4	1
	2	$\text{C}_{14}\text{H}_{11}\text{O}_3$	227.0711	0.3	3
	3	$\text{C}_{16}\text{H}_{13}\text{O}_5$	285.0737	-2.5	5
Phenanthrene‡	2	$\text{C}_{17}\text{H}_{11}\text{O}_3$	263.0699	-0.8	20
	3	$\text{C}_{19}\text{H}_{13}\text{O}_5$	321.0756	-0.6	17
	4	$\text{C}_{21}\text{H}_{15}\text{O}_7$	379.0813	-0.4	8
OCH_3 -phenanthrene†	2	$\text{C}_{18}\text{H}_{13}\text{O}_4$	293.0790	-2.3	8
Fluoranthene§	3	$\text{C}_{21}\text{H}_{13}\text{O}_5$	345.0744	-1.8	4
	4	$\text{C}_{23}\text{H}_{15}\text{O}_7$	403.0842	2.5	3
Fluorenone¶	2	$\text{C}_{16}\text{H}_9\text{O}_4$	265.0517	1.8	5
OCH_3 -fluorenone¶	1	$\text{C}_{15}\text{H}_9\text{O}_3$	237.0568	1.7	1
Anthraquinone¶	2	$\text{C}_{17}\text{H}_9\text{O}_5$	293.0455	0.6	8
Dibenzofuran and/or naphthofuran	1	$\text{C}_{13}\text{H}_7\text{O}_2$	195.0456	1.1	18
	2	$\text{C}_{15}\text{H}_9\text{O}_4$	253.0506	0.6	35
	3	$\text{C}_{17}\text{H}_{11}\text{O}_6$	311.0576	2.1	28
	4	$\text{C}_{19}\text{H}_{13}\text{O}_8$	369.0588	-2.0	8
Benzonaphthofuran	2	$\text{C}_{19}\text{H}_{12}\text{O}_4$	303.0692	3.6	5
	3	$\text{C}_{21}\text{H}_{13}\text{O}_6$	361.0738	2.7	7
Xanthone	1	$\text{C}_{14}\text{H}_7\text{O}_3$	223.0397	0.3	33
	2	$\text{C}_{16}\text{H}_9\text{O}_5$	281.0433	-1.6	32
Benzothiophene	2	$\text{C}_{11}\text{H}_7\text{O}_3\text{S}$	219.0122	0.6	9
	3	$\text{C}_{13}\text{H}_9\text{O}_5\text{S}$	277.0134	-3.5	1
Dibenzothiophene and/or naphthothiophene	2	$\text{C}_{15}\text{H}_9\text{O}_3\text{S}$	269.0244	-2.7	5

*Benzene tricarboxylic acid methyl ester is normalised to 100.

†Methoxy compounds may result from the reaction of phenolic compounds with diazomethane. Methoxyphenanthrene may be derived from the reaction of fluorenone with diazomethane¹⁴.

‡Although the phenanthrene ring is not affected during oxidation, anthracene is oxidised to anthraquinone⁶.

§Methyl esters of pyrene carboxylic acids show very similar mass spectra, however, the pyrene ring is extensively degraded by present procedure⁸.

¶These compounds might be oxidised further to benzene-carboxylic acids in our experimental conditions.

||It is probable that our estimates of the first two dibenzofurans are somewhat high because of contribution from fragments of xanthenes which lose both CO and OCH_3 to give the corresponding dibenzofuran ions.

Illinois bituminous coal was finely powdered, heated with 12 N HCl and washed. The same procedure was repeated three times, and the coal dried at 120 °C. The sample contained 71.33% carbon.

Table 2 N-heterocyclics found in the photochemical oxidation of coal in alkali solution, identified as methyl esters of carboxylic acids

Nucleus	Number of -COOCH ₃	Elemental composition	Precise mass (M-OCH ₃) ⁺		Relative* abundance (±15–20%)
			Observed	Deviation × 10 ³	
Pyridine	3	C ₁₀ H ₈ O ₅ N	222.0408	0.5	3.0
	4	C ₁₂ H ₁₀ O ₇ N	280.0488	3.2	3.5
Quinoline and/or isoquinoline	1	C ₁₀ H ₆ ON	156.0412	−3.7	0.5
	2	C ₁₂ H ₈ O ₃ N	214.0468	−3.5	2
Carbazole†	1	C ₁₃ H ₈ O ₃ N	194.0598	−0.7	10
	2	C ₁₅ H ₁₀ O ₅ N	252.0660	0.0	9
	3	C ₁₇ H ₁₂ O ₇ N	310.0708	−0.6	5

*Benzene tricarboxylic acid, which is the most abundant aromatic acid in this oxidation product, is normalised to 100.

†Carbazole is sensitive to oxidation with oxidising agents; however, this nucleus is stable during photochemical oxidation as shown by our own experiments with authentic carbazole and N-ethyl carbazole.

Demineralised fine powdered coal was suspended in either 10% HCl or 5% KOH aqueous solution, and oxidised with air by the radiation of a high pressure mercury lamp for 7–10 d at 40–50 °C.

this view but rather the generally accepted idea that coal is predominantly an aromatic material.

We have identified 35 aromatic carboxylic acids resulting from the oxidation of bituminous coal with aqueous Na₂Cr₂O₇ (Table 1). This oxidation procedure was chosen as it had been shown⁶ that it will oxidise substituted polycyclic aromatic compounds in good yields to their corresponding polynuclear carboxylic acids with a minimum of ring degradation. It seemed probable, therefore, that aliphatic and alicyclic linkages between aromatic units in coal would be broken, with the formation of carboxylic groups and the release of smaller aromatic units.

Illinois bituminous coal (3.6 g Peabody coal from seam No. 2) was heated at 250 °C in 120 ml 0.4 M Na₂Cr₂O₇ in a stainless steel autoclave for 36 h. The aromatic acids formed were isolated by a method similar to that of Friedman *et al.*⁶. The coal, which was almost completely insoluble in either

aqueous or organic solvents, was completely solubilised with the resulting acids weighing 53% of the original weight of coal. The acids were converted to methyl esters with diazomethane to increase their volatility for subsequent mass analysis. Individual compounds were identified by a time of flight mass spectrometer with a temperature-programmed solid inlet⁷, and verified by a precise mass determination with a high resolution mass spectrometer-computer combination. The mass spectra of methyl esters of aromatic carboxylic acids are relatively simple. The most abundant peak in practically all cases is formed by the loss of a methoxy group from the molecular ion (M-31)⁺ (refs 8–10). In addition, sizeable molecular ions and (M-COOCH₃)⁺ are observed and were used to verify the identification of each compound. Relative abundances were estimated from an integration of the base peak of each compound during the time that a sample was completely volatilised in the mass spectrometer.

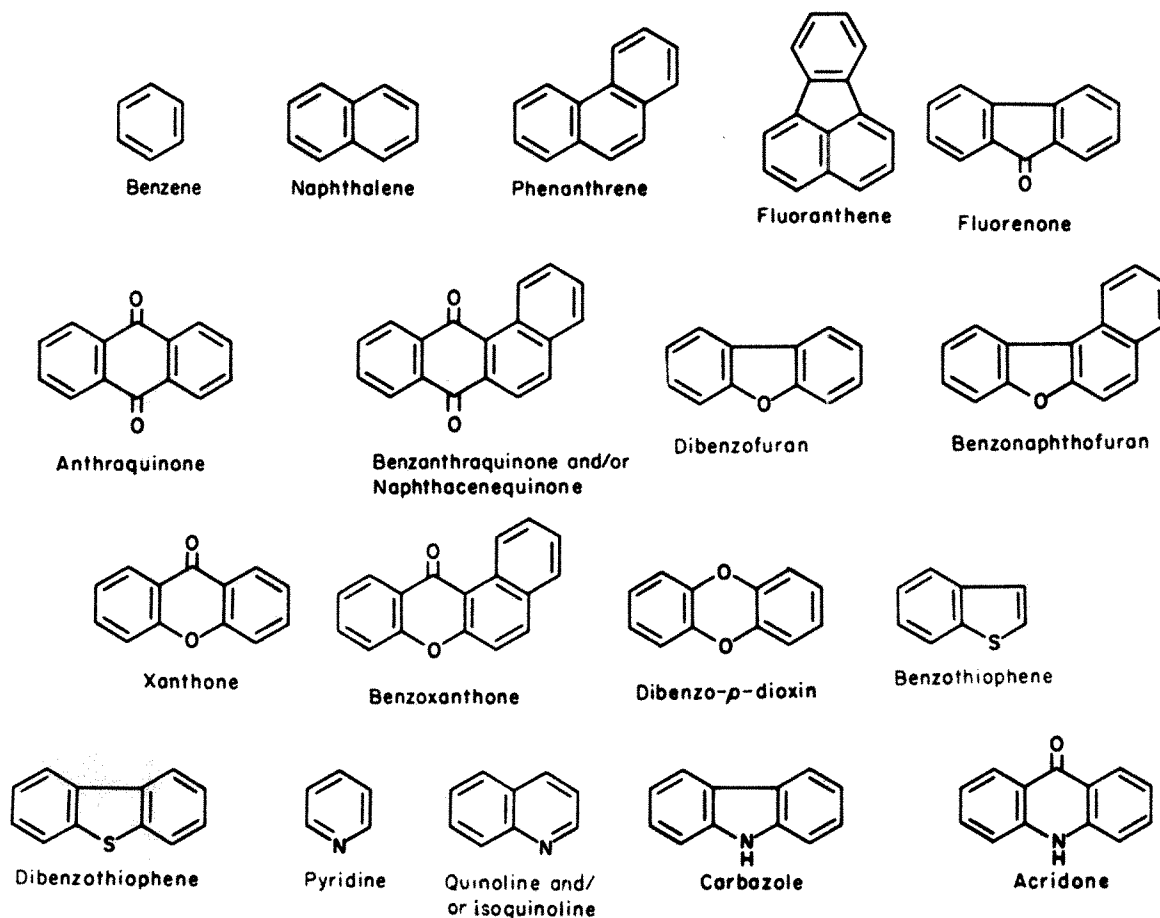
**Fig. 1** Aromatic units indigenous to coal.

Table 1 summarises the data for the methyl esters of the 35 aromatic acids which were positively identified. Another 16 oxygen-containing ions in considerable yield were observed in the mass spectra of the acid fraction but not identified.

In addition several series of interesting aromatic derivatives were observed in the chemically neutral fraction. These included dibenzo-*p*-dioxins, benzoxanthones, benzantraquinones and acridones. These compounds exhibit an abundant M^+ as the base peak of the spectra¹¹. Amphoteric nitrogen-containing acids have not been isolated from the dichromate oxidation product because of some experimental difficulties. Seven nitrogen-containing aromatic acids produced in a photochemical oxidation were, however, identified (Table 2). Figure 1 shows the structural formulae of the aromatic units we believe to have been indigenous to the coal. Fluorenone and anthraquinone may have been produced by oxidation of fluorenes and anthracenes, respectively.

Of particular interest are the heterocyclic units of Fig. 1 with O, S, and N in ring systems. Little is known about the chemical state in coal of these important elements^{12,13} which are particularly troublesome in processes for conversion of coals to liquids and gases. Some of the aromatic units (Fig. 1) have been observed in tars produced by pyrolysis or other high temperature processes but it was not known whether these were indigenous or were produced in the process. For example benzo- and dibenzothiophene are formed when sulphur is heated with a variety of hydrocarbons. It is not likely that such compounds were formed in our low temperature aqueous oxidation experiments and thus thiophene derivatives must be indigenous to coal.

The complexity of the observed aromatic acids—with up to five carboxylic groups on benzene and up to four on polycyclic aromatics—suggests a high degree of aliphatic or alicyclic linkages between aromatic units. Such linkages are more readily oxidised than are the aromatic rings.

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First fossil records of cephalopod statoliths

THE class Cephalopoda is represented by over 10,000 fossil species and about 1,000 living species. Almost the entire fossil record so far described consists of external or internal calcareous shells of the Ammonoidea, the Nautiloidea and

some members of the Coleoidea. The subclass Coleoidea includes all but three (*Nautilus* spp.) of the living species of cephalopods, and is represented in the fossil record largely by the order Belemnitida which is important from the early Jurassic to the Eocene. Another coleoid order, the Sepioidea, including the living *Sepia* and *Spirula* have internal calcareous shells, and have left traces from the Upper Jurassic (*Voltzia*) to the present. The three remaining coleoid orders, the Teuthoidea, the Vampyromorpha and the Octopodida include all the remaining living cephalopods, comprising 29 teuthoid or squid families, one vampyromorph family and 12 octopod families.

With the exception of *Argonauta*, the 42 extant cephalopod families have no calcareous shells and such fossils as are known consist of body imprints, beaks, hooks, radulas, chitinous pens and ink sacs. Of these, beaks¹, body imprints and pens² have the most potential in relating fossils to living forms but are too few at present to allow the construction of even the most modest of phylogenetic trees.

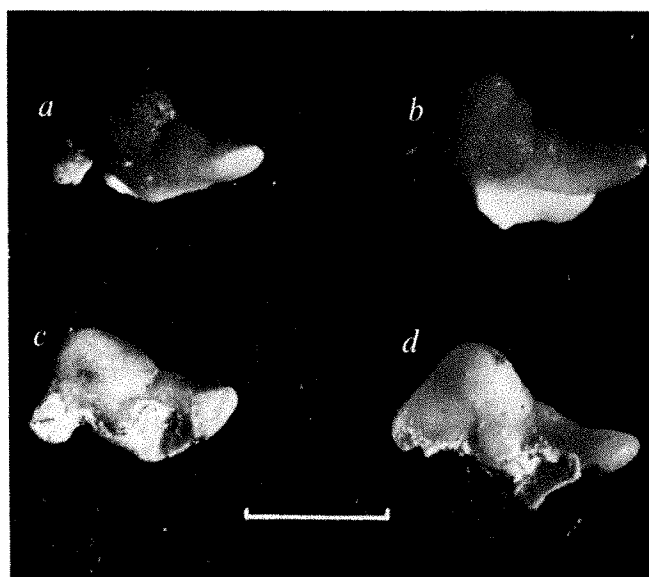


Fig. 1 *a*, The posterior and, *c*, anterior surfaces of, respectively, right and left statoliths from a Miocene deposit (Olcese Sand, California); *b*, the posterior and, *d*, anterior surfaces of respectively, right and left statoliths of a living oceanic squid, *Todarodes sagittatus*. Scale, 1 mm.

There are, however, other hard structures in cephalopods, which have so far been neglected in the study of both living and fossil forms. These are known as statoliths (= otoliths of some authors), small, paired calcareous stones with a complicated shape very characteristic of the Sepioidea and Teuthoidea, although the basic pattern varies within those groups.

Statoliths of at least one species of cephalopod are composed of aragonite, a stable form of calcium carbonate commonly found in fossils, including the fossilised otoliths of fish. It is, therefore, not surprising that cephalopod statoliths should be discovered in Cainozoic deposits. It is, however, of particular interest as it offers a possibly definitive technique for unravelling Cainozoic cephalopod phylogeny. Figure 1*a-d* compares anterior and posterior views of left and right statoliths from a Miocene teuthoid with those from an individual of the extant *Todarodes sagittatus*. Though the latter (Fig. 1*b* and *d*) are white and slightly translucent, the fossils (Fig. 1*a* and *c*) are light brown and more opaque. They will be described in more detail elsewhere.

An examination of unidentified fossil otoliths and statoliths has revealed a number of squid statoliths from North American strata. To date they have been found in the Pleistocene of California (Timms Point Silt), the Pliocene of California (Lomita Marl and Pico Formation), the Miocene of California (Olcese Sand), Florida (Chipola Formation), Jamaica (Bowden Formation), and Virginia (Yorktown Formation), and the Oligocene of Mississippi (Glendon Limestone). In one Pliocene deposit (Lomita Marl), 185 of 24,299 otoliths and statoliths recovered from approximately one ton of fossiliferous matrix represented at least three kinds of squid species. Rare fossils, very similar to modern ommastrephids *Plesioteuthis*, are known from the Upper Jurassic, suggesting that statoliths are likely to be found in strata older than the Cainozoic.

Statocysts of living cephalopods have been described by several authors³⁻⁷ but no detailed description or comparative study of the hard calcareous statolith (= otolith) has so far been published.

M. R. Clarke (unpublished) found that the statoliths of *Ommastrephes* were composed of aragonite, and in another study attempted to relate growth to the rings seen in the statoliths of *Todarodes sagittatus*. Unaltered aragonite fossils (teleost fish otoliths, mollusc shells, and so on) are abundant throughout the Cainozoic, and sometimes afford the only method of identifying faunal components^{8,9}. Indeed, fish otoliths have proved very useful in tracing the history of Recent genera, and there seems every possibility that statoliths will help clarify the relationships of Recent families, and possibly even genera, of the Cephalopoda. A study of the variation of statoliths in Recent families of cephalopods is now in progress, and it is already evident that within the limits of the general pattern the form varies considerably between and within families.

Fossil cephalopod statoliths should provide not only a means of studying phylogeny but, in addition, the relative numbers of statoliths of different families should provide an indication of the relative importance of the various families living at a particular locality and time.

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fluctuation field¹. A two-dimensional array study operated over a wide area of southern Australia has detected anomalous effects at the eastern edge of the seismically active Flinders Ranges². Various interpretations have been suggested for the Flinders anomaly³ and interpretations of the accompanying seismicity³ have been given in terms of plate tectonics⁴. Independent seismic evidence⁵ indicates that there is no abrupt step in crustal and upper mantle structure from west to east across the Flinders Ranges, though there may be a gradual change. A magnetometer array in south-eastern Australia has detected anomalous effects in southern Victoria⁶, and the interpretation of a subsequent study has located the anomaly more exactly (F. E. M. L., unpublished), placing the conductor causing it under or near the Otway Ranges, another region of Australian continental seismicity. Consequently, all the major continental conductivity anomalies so far discovered in Australia are in regions of earthquake activity (Fig. 1).

The earthquakes plotted in Fig. 1 occurred between 1897 and 1972, and the distribution of observatories on the continent over this time has probably influenced the seismicity pattern shown. Several major earthquake zones are apparent, though on a world scale the seismicity of the Australian continent is low.

It is possible that the electrical conductivity anomalies and seismic zones are geographically related. Shallow conductivity anomalies could arise because fracturing has broken up the surface rocks, allowing continuity in saline ground waters to provide a conducting channel. This mechanism is, however, not entirely satisfactory for the Otway Anomaly, which may extend deeper than the seismic zone.

Beneath fracture zones, motion along fault planes presumably occurs by some type of ductile flow. Such ductile shearing of rock may increase greatly its electrical conductivity and produce a deep electrical conductivity anomaly, either by causing a change in rock fabric⁷ (for example the presence of a graphite schist has been postulated to explain a major conductive structure in North America⁸), or simply by heating. The rheological process of ductile flow beneath continental earthquake zones may involve the conversion of considerable quantities of mechanical energy to heat.

Magnetic fluctuations may in future be observed over the more northern centres of Australian seismicity (see Fig. 1) which have not yet been covered by magnetometer array studies. Because of present interest in the thermal balance of active faults⁹, heat flow data are being collated, although heat flow values would not necessarily be expected to be high: increased conductivity could result from quite a minimal increase in temperature at depth were that temperature already near the melting point, thus forming a partial melt.

If a general relationship between seismic activity and electrical conductivity anomalies is established there may be several implications for projects of earthquake prediction. The first is that deep electrical conductivity may vary both before and after an earthquake, as a consequence of associated variations in the ductile flow. (I do not mean to imply that such variations would be detected by a magnetometer array; perhaps controlled-source, electrical methods would be most suitable.) The second implication is that any observations of magnetostrictive changes in the Earth's static magnetic field may need to be corrected for non-uniform magnetic fluctuations arising from local conductivity anomalies. Such corrections may be possible if magnetostrictive observations are taken on an array basis, and full geomagnetic induction tensors¹⁰ are estimated for each observing site.

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Electrical conductivity anomalies and continental seismicity in Australia

WHERE the Upper Mantle Project 'geotraverse' line in western Australia crosses seismic zones anomalous effects have been found in the vertical component of the magnetic

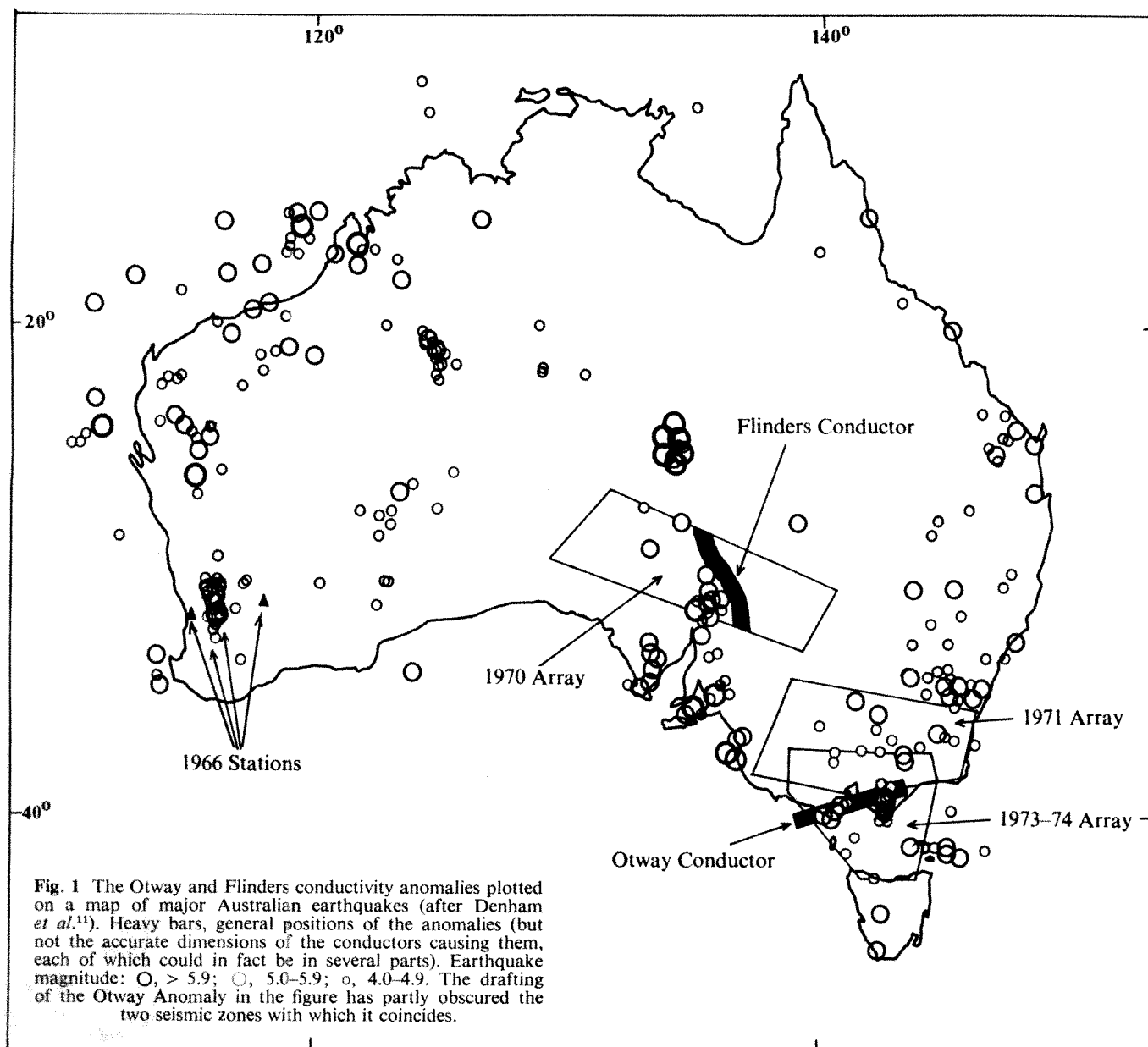


Fig. 1 The Otway and Flinders conductivity anomalies plotted on a map of major Australian earthquakes (after Denham *et al.*¹¹). Heavy bars, general positions of the conductors (but not the accurate dimensions of the conductors causing them, each of which could in fact be in several parts). Earthquake magnitude: \bigcirc , > 5.9 ; \bigcirc , $5.0-5.9$; \circ , $4.0-4.9$. The drafting of the Otway Anomaly in the figure has partly obscured the two seismic zones with which it coincides.

The Bureau of Mineral Resources provided maps of Australian earthquakes.

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Tyndall figures at grain boundaries of pure ice

LIQUID figures produced by internal melting in ice are called Tyndall figures (see refs 1-3). I report here the

formation and morphology of Tyndall figures at grain boundaries in pure ice.

I produced Tyndall figures at grain boundaries by focusing radiation from a small lamp into an area of $2 \times 2 \text{ mm}^2$ in an ice specimen which had grown from degassed, distilled and deionised water in stainless steel containers, and which had reached melting point as indicated by veins of water forming along the intersections of three grain boundaries⁴.

Two Tyndall figures with a vapour cavity were formed at a grain boundary (Fig. 1a). The vapour cavities—the black parts in Fig. 1a—are produced because of the density difference between ice and water¹. The Tyndall figures are inclined to the plane of the figure. The dashed arrows and the solid arrow (Fig. 1a) show the veins of water and the intersection of the melt grain boundary and the specimen surface, respectively. The surface of the melt boundary is not flat.

Fig. 1a shows that small perturbations are formed at the periphery of the Tyndall figures. Such perturbations were observed at the periphery of disk-shaped Tyndall figures formed in the basal plane. When the figures in the basal plane developed to a certain size, they became truncated cones and the perturbations began to be nucleated at the edge where the side face and the planar plane of the figures intersected³. On the other hand, the perturbation of

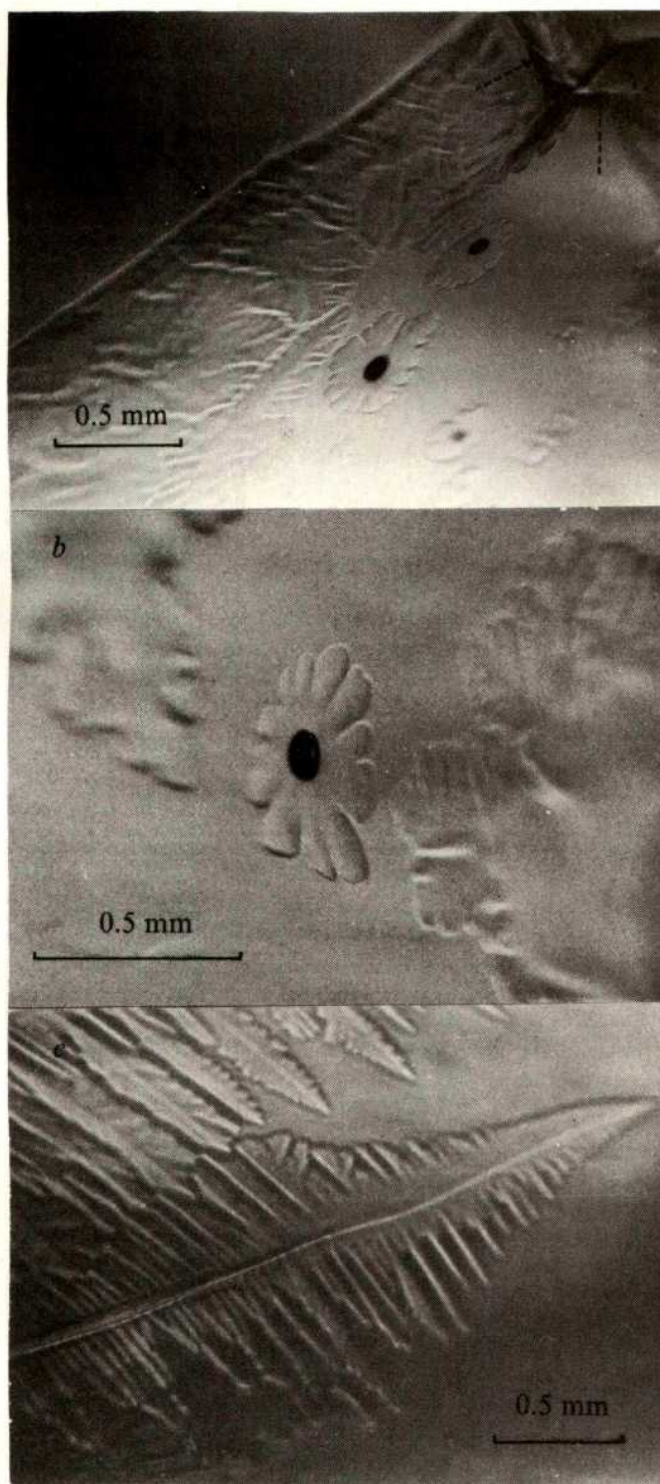


Fig. 1 *a*, Tyndall figures in a grain boundary; *b*, a 12-branched Tyndall figure; *c*, shape of a large branch of a Tyndall figure.

Tyndall figures at grain boundaries were observed even at an initial stage of their growth. Considering that the periphery of the figures at grain boundaries is always sharp because the angle where the figures meet the boundaries is 32° in the liquid phase (see ref. 4), I conclude that this kind of perturbation is nucleated at sharp edges.

During the growth of the Tyndall figures at the grain boundaries their shape changed, though some perturbations disappeared and others were nucleated. When the growth rate was large, the perturbations were sharp and their number increased. On the other hand, when the growth

rate was small, the perturbations were wide and their number decreased. The minimum number of perturbations that I observed was 12 (Fig. 1*b*).

Twelve-branched snow crystals, though rarely observed in nature, have been reported by some authors. Nakaya⁶ has examined them and concluded that they comprise two hexagonal crystals, one attached to the other. Kobayashi and Furukawa⁷ have explained their formation in terms of rotation twinning. This mechanism cannot be applied, however, to their formation because, although the orientation of adjoining grains in Fig. 1*b* could not be measured, it is clear from other observations that the morphology of Tyndall figures at grain boundaries does not depend on the orientation of the adjoining grains. Therefore, it is necessary to consider a new mechanism for the formation of 12-branched Tyndall figures.

When Tyndall figures grow large and the radiation on them becomes inhomogeneous, one of the perturbations grows preferentially and a large branch forms (Fig. 1*c*). The direction of the branch changes slightly but is usually straight. The configuration of the twigs on the branch shown in Fig. 1*c* is different from that of Tyndall figures formed in the basal plane. The angle between twigs and branches of Tyndall figures formed in the basal plane is 60° ; on the other hand, the angle between Tyndall figures at grain boundaries is 60° , 75° and 90° . The configuration of twigs on Tyndall figures at grain boundaries is similar to that observed on Tyndall figures formed in the crystallographic plane perpendicular to the basal plane (see ref. 2). This similarity may occur because the non-basal plane is less closely-packed than the basal plane.

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Photoelectrolysis of water using semiconducting TiO_2 crystals

FUJISHIMA and Honda¹ have indicated that TiO_2 can be used in a photoelectrochemical cell to decompose water with band-gap light ($h\nu \geq 3.0 \text{ eV}$). This process, termed photoelectrolysis, could have important implications for solar energy conversion and the 'hydrogen economy' if use can be made of visible light.

To understand some of the basic aspects of photoelectrolysis, we have studied the phenomena with rutile single crystals as a function of light intensity, internal cell potential and resistance, pH, and cathode material. Rutile crystal wafers (*c* axis in the wafer plane) were reduced in H_2 to produce an n-type conductivity of $0.3 \Omega^{-1} \text{ cm}^{-1}$, and were then polished, etched, and fabricated into electrodes.

TiO_2 electrodes were studied in two types of cell in which the cathodes were usually platinised platinum foil (2.2 cm^2). The first type was a homogeneous cell in which the TiO_2 and Pt electrodes were immersed in the same electrolyte. The second cell consisted of two separate compartments connected by a KCl-agar salt bridge so that the electrodes could be immersed in different electrolytes. The TiO_2 crystal was illuminated with broad band ($3,000\text{--}4,000 \text{ \AA}$) near-ultraviolet light with an intensity of

26 mW cm⁻². This irradiation was varied over four orders of magnitude with neutral density filters having absorbance (*A*) values of 1, 2 and 3.

The current-voltage characteristics of a TiO₂ crystal (area 0.76 cm², thickness 0.52 mm) were measured as a function of light intensity in the homogeneous cell using 1 N phosphate buffer (pH = 6.5). With cathodic (forward) bias, the *i*-*V* curves showed typical diode behaviour. In the third quadrant (cathodic bias and current) the results were independent of light intensity; in the second quadrant (cathodic bias and anodic current) the results depended on light intensity in the usual manner.

The *i*-*V* data for anodic (reverse) bias is shown in Fig. 1. In the dark and at low illumination levels (*A* = 3 and 2), the

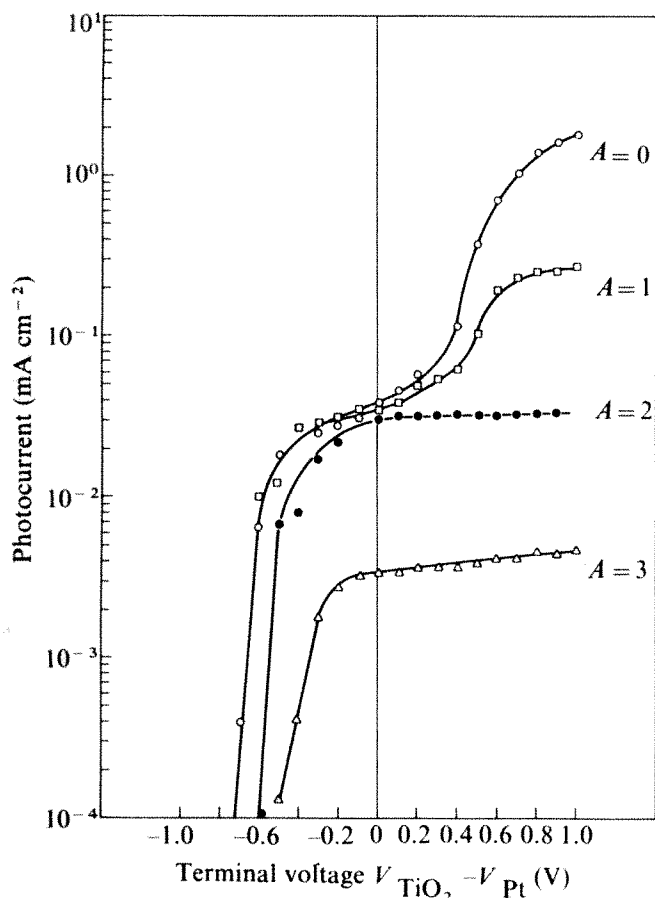


Fig. 1 Current-voltage characteristics for a TiO₂ crystal electrode in 1 N phosphate buffer (0.33 M Na₂HPO₄ + NaH₂PO₄) as a function of light intensity (homogeneous cell). The dark current is essentially zero (< 10⁻⁴ mA) with anodic bias; *A* = 0 corresponds to 26 mW cm⁻².

curves again show normal diode behaviour. The current is determined by the availability of holes in the TiO₂, and this is proportional to light intensity. At higher intensities, however, two important changes occur: (1) when the bias is between zero (short circuit) and +0.3 V the photocurrent saturates at light intensities ≥ 0.26 mW cm⁻² (*A* = 2), and (2) above a critical bias of $\sim +0.3$ –0.4 V the photocurrent at *A* = 0 and 1 undergoes a sharp increase with bias accompanied by visible H₂ and O₂ evolution at the cathode and anode, respectively. At about +1.0 V, the photocurrent again saturates with respect to bias, but is now approximately proportional to light intensity.

The electrode potentials (against a standard calomel electrode (SCE)) for both TiO₂ and Pt are plotted in Fig. 2. As expected, the Pt potential is independent of light intensity, and above the critical bias exhibits the normal value for H₂ evolution.

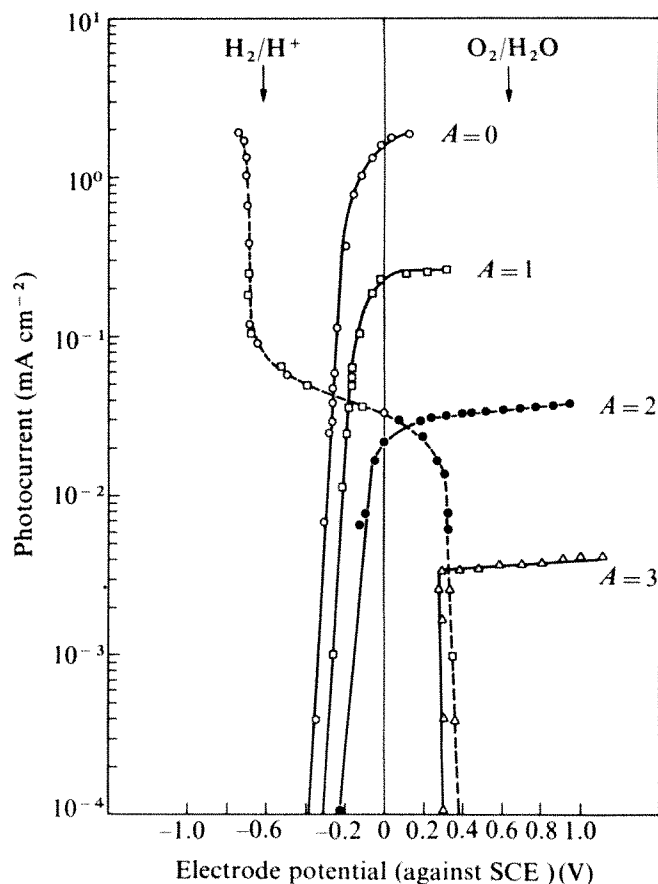


Fig. 2 TiO₂ and Pt electrode potentials as a function of light intensity in the homogeneous cell (1 N phosphate buffer). The normal H₂ and O₂ evolution potentials are indicated. ---, $V_{Pt} - V_{SCE}$; —, $V_{TiO_2} - V_{SCE}$.

The TiO₂ electrode potential depends on light intensity. At 26 mW cm⁻², the O₂ evolution potential is -0.26 V; the normal value is +0.62 V. Lower illumination intensities produce smaller negative shifts of the O₂ potential. For H₂ evolution to occur with zero bias, the potential for O₂ evolution on TiO₂ must be shifted to the negative side of the H₂ potential (a shift of -1.23 eV in a homogeneous cell).

The necessity for anodic bias to produce gaseous evolution can be understood by considering an energy balance for the absorbed photon². For band-gap photons ($h\nu \geq E_g$):

$$E_g + E_b = V_B + (\Delta G/2F) + V_H + iR + (E_c - E_f) + \eta_{Pt} + \eta_{TiO_2} \quad (1)$$

where E_g is the band gap (3.0 eV), E_b the bias, V_B the potential barrier of the TiO₂-electrolyte junction (~ 0.8 eV), $\Delta G/2F$ is the free energy per electron for H₂O decomposition (1.23 eV), V_H is the drop across the Helmholtz layer in the electrolyte (~ 0.05 eV), iR is the ohmic loss (~ 0.05 eV), $(E_c - E_f)$ is the energy difference between the conduction band and the Fermi level in TiO₂ (~ 0.2 eV), and η_{Pt} and η_{TiO_2} are the respective electrode overpotentials (~ 0.1 eV for Pt). The estimate for the potential barrier (V_B) was determined from a Schottky-Mott plot of $1/C^2$ against E_b , where C is the capacitance of the depletion layer^{3,4}. Values indicated for the other terms in equation (1) are either well known or were calculated from basic considerations.

In equation (1), the input energy requirements for gaseous evolution are 3.3–3.5 eV. All the terms on the right, except for η_{TiO_2} , account for ~ 2.4 eV. Thus, the O₂ overvoltage, η_{TiO_2} , is estimated to be ~ 0.9 –1.1 eV. This results from kinetic limitations on the rate of hole injection from the TiO₂ space charge layer into the electrolyte.

Table 1 Power balance for photoelectrolysis

Cell*	Electrolyte	Internal cell resistance (Ω)	Bias (V)	Bias power (mW)	H ₂ evolution† (output chemical power) (mW)	Optical conversion efficiency‡ (%)
Homogeneous	1 N phosphate buffer	380	1.0	0.99	1.47	2.4
			0.9	0.77	1.26	2.5
			0.8	0.55	1.02	2.4
			0.7	0.36	0.77	2.1
			0.6	0.20	0.50	1.5
			0.5	0.095	0.28	1.0
			1.0	1.40	2.07	3.4
	1 N phosphate buffer	215	0.8	0.84	1.56	3.7
			0.6	0.32	0.80	2.4
			1.0	1.37	2.03	3.4
	0.1 N KOH	220	0.8	0.86	1.60	3.8
			0.6	0.38	1.06	3.2
	0.2 N H ₂ SO ₄	65	1.0	1.68	2.49	4.1
			0.8	0.94	1.75	4.1
			0.6	0.33	0.81	2.4
Differential pH	0.1 N KOH (TiO ₂)/ 0.2 N H ₂ SO ₄ (Pt)	300	0	0	1.87	9.5¶
			-0.2	-0.16§	1.16	6.7
			-0.4	-0.12§	0.46	2.9
			+0.2	0.33	2.43	10.7
			+0.4	0.74	2.73	10.1

* Optical near-ultraviolet power input is 19.7 mW (3,000–4,000 Å).

† Output chemical power is based on heat of combustion of H₂ to H₂O(l), and equals $(3.543) \times r$ where $r = \text{cm}^3 \text{H}_2 \text{h}^{-1}$.

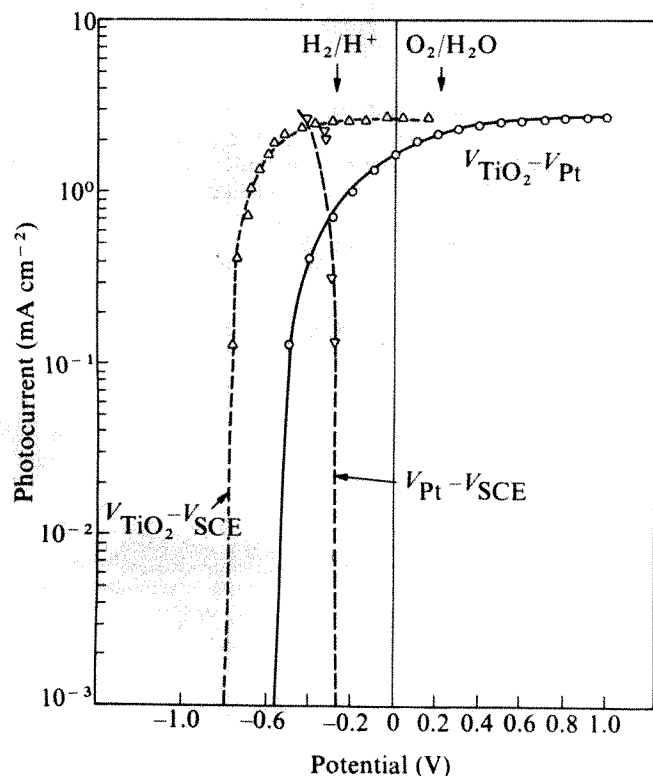
‡ Defined as (chemical power out – electrical power in)/(optical power in).

§ Electrical power withdrawn from cell.

¶ Efficiencies for the differential pH cell do not take into account the energy required to maintain the differential pH during photoelectrolysis.

One intriguing way of supplying the anodic bias is to create a protonic concentration gradient between the Pt and TiO₂ electrodes. This was done in the differential cell by placing the TiO₂ electrode in 0.1 N KOH and the Pt electrode in 0.2 N H₂SO₄. The i - V characteristic of this cell is shown in Fig. 3; at zero bias, vigorous evolution of H₂ and O₂ occurs. Gaseous evolution can be maintained even with a cathodic bias, meaning that both chemical and electrical power can be withdrawn from the cell.

Fig. 3 The i - V characteristic and electrode potentials for TiO₂ and Pt in a differential pH cell (0.1 N KOH/0.2 N H₂SO₄). The normal H₂ and O₂ evolution potentials are indicated for each compartment.



Although the rest potential of the cell is 0.78 V, no current flows in the dark under short circuit or anodic bias conditions. The electrode potentials are also shown in Fig. 3. The Pt electrode shows the normal H₂ evolution potential at all current densities. The potential for O₂ evolution on illuminated TiO₂ is shifted by ~ -1.0 V.

A power balance was made for both types of cell by measuring the input electrical and optical power, and the output chemical power (that is, the H₂ evolution rate). The results are summarised in Table 1. They show that (1) the optical conversion efficiency (OCE) is maximised at $\sim +0.8$ V for the homogeneous cell (3–4%) and at $+0.2$ V for the differential cell ($\sim 11\%$); (2) the OCE increases with decreased internal cell resistance but is unaffected by pH; and (3) the H₂ evolution rate is very sensitive to bias between $+0.4$ and 1.0 V. Additional studies of cell parameters show that the OCE increases with decreased light intensity, and that the critical bias using other metal cathodes is increased by the difference between their H₂ overvoltage and that of platinised Pt.

In summary, these studies show that the necessity of a bias for H₂ evolution arises from an O₂ overvoltage of about 1 V at the TiO₂ anode; the bias can be applied either with an external voltage or by creating a concentration gradient within the cell. The viability of photoelectrolysis with anodic bias must be examined further, especially for the cases where smaller band gap semiconductors are contemplated for improved solar absorptivity.

I note that other workers⁵ have also measured optical conversion efficiencies for TiO₂ which are comparable with the values reported here.

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Technique for the study of diffusion of large molecules in polymers based on infrared microdensitometry

THE diffusion of small molecules (in the gaseous, vapour or liquid phases) through polymeric matrices has been the subject of much study¹, and the techniques for such studies are, by and large, well established. Similar studies in the case of large molecules (broadly speaking, those forming solids at room temperature) in polymers, including polymeric self-diffusion, have been more rare²⁻⁵. Existing techniques for measurements of the diffusion of large molecules in synthetic polymers rely mainly on radioactive labelling of the diffusants. Nuclear magnetic resonance (NMR) has been used⁵, but is limited to measurements of diffusion in the melt, and also to relatively high diffusion rates⁵. Measurements involving radioactively labelled diffusants have two major shortcomings, in addition to the necessity of having to obtain the required labelled material: they fail with diffusants which are surface active with respect to the polymer², and they may involve significant interfacial resistance which the method does not reveal^{1,3}.

We have developed a simple technique for the measurement of the translational diffusion of large molecules in polymeric matrices, based on microdensitometry in the infrared. Essentially, a step function in the concentration of the diffusant is set up within the bulk matrix, and diffusion is allowed to occur at the required pressure and temperature. At some known time the diffusion is effectively terminated by quenching, and a thin slice through the composite centre is removed and scanned in the microdensitometer (Fig. 1).

To obtain a measure of the concentration of the diffusant it is necessary that it absorb at some infrared frequency unaffected by the bulk polymer, and the scanning of the thin section is, therefore, carried out at that frequency. A commercial double-beam infrared spectrometer has been adapted for the purpose, and is capable of achieving a resolution of better than 100 μm in lateral scan. Figure 2 shows the expected and observed concentration profiles for a typical run. The value of the diffusion coefficient D , is

readily evaluated from the shape of the profile⁶. It is also possible to evaluate the concentration dependence of D , where it is significant. Interface resistance, if any, is revealed by discontinuities in the broadened concentration profile⁶. Values of D in the range 10^{-5} – 10^{-10} $\text{cm}^2 \text{s}^{-1}$ may be obtained in times of the order of 1–30 d, and the estimated errors are of the order of 15%.

We have studied the diffusion of various long-chain, n -alkyl amides in low density polyethylene at about the melting temperature. The dependence on temperature and chain length is summarised in Fig. 3. The discontinuity in the slope of the Arrhenius plot as the temperature is raised is probably the result of the breakdown of the crystalline lamellae in the polythene, which at temperatures below

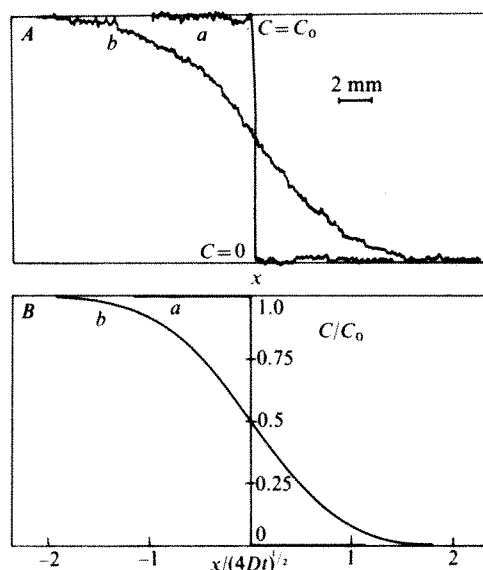


Fig. 2 Concentration profiles. A, observed profiles: a, $t \approx 0$ (initial step function; $t = 54$ s, $C_0 = 0.5\%$ stearamide in low density polythene (LDPE), weight ratio); b, after time $t(t = 5.22 \times 10^4$ s., $T = 130^\circ \text{C}$; C_0 as in (a)). Both profiles scanned at $1,660 \text{ cm}^{-1}$, an amide absorption frequency. B, calculated profiles⁶.

about 80°C comprise some 60% of the polymer; this leads to a decrease in the tortuosity of the path taken by the amide chains in the amorphous polymer regions, over and above the usual temperature dependence.

The diffusional behaviour of longer molecules in polymeric matrices is being explored.

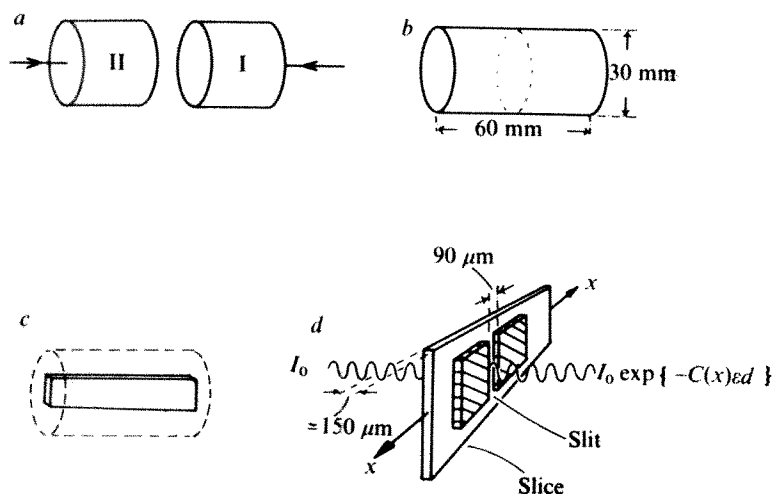


Fig. 1 Outline of experimental procedure: a, Specimen I—pure polymer and specimen II—polymer + diffusant, dispersed uniformly (about 0.1–1% w/w); b, specimen I and II joined together; c, slice removed from centre of composite; d, slice scanned in x direction for concentration profile. e , Extinction coefficient of the diffusant at the chosen infrared wavelength, and the concentration $C(x)$ as x varies is recorded directly (Fig. 2) using a linear-log converter.

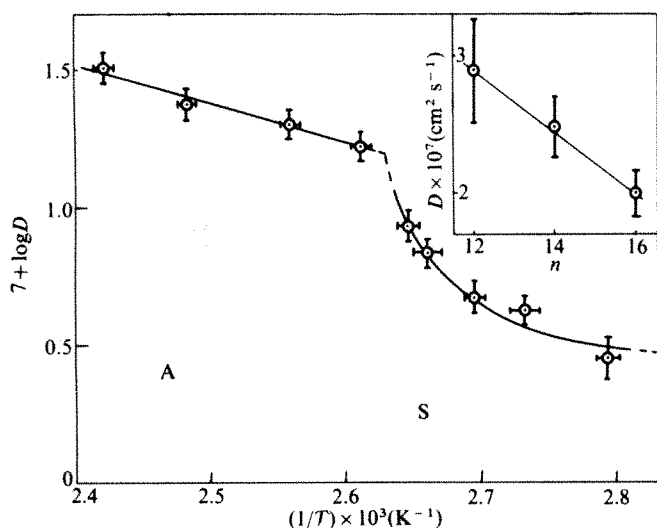


Fig. 3 Variation of the diffusion constant, D , of stearamide in LDPE as a function of temperature, T , about the melting region of the polymer: A, amorphous regime; S, semicrystalline regime. Inset, Diffusion constants, D , of $\text{CH}_3(\text{CH}_2)_n\text{CONH}_2$ in LDPE as a function of n , at 118°C . D decreases as diffusant length increases.

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Palaeotemperatures from tree rings and the D/H ratio of cellulose as a biochemical thermometer

DENDROCHRONOLOGY can provide sequences of tree rings from various parts of the world. The wood making up these rings can be accurately dated to ± 1 yr. Samples can be obtained going back a considerable time, for example wood from *Pinus aristata* (Bristle Cone Pine) trees can be obtained which goes back beyond 9,000 yr BP. If the isotopic ratios of the elements in the individual constituents in the wood contain a record of past climate, it may enable a curve of a climatic variable like temperature to be determined on a time base accurate to ± 1 yr.

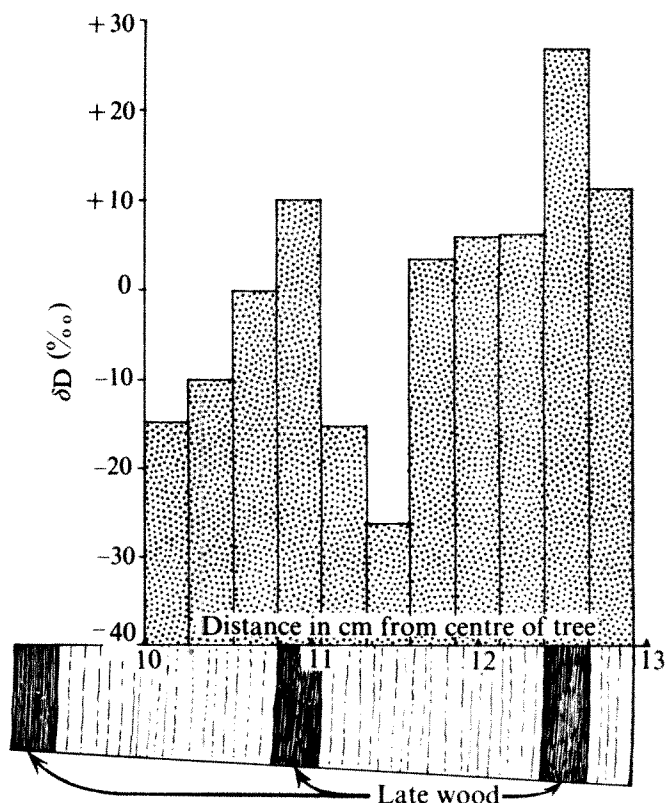
One might expect the biochemical pathways leading from CO_2 and water to the compounds that make up the wood to have isotopic effects. The question is whether the isotopic effects have significant temperature coefficients. If they do, one would have in effect a "biochemical thermometer". Here we report the existence of such a biochemical thermometer.

To study how the isotopic composition of the constituents of wood varies with temperature we have studied wood laid down during the course of one year by a *Pinus radiata* (Monterey Pine) tree growing in Hamilton, New Zealand. This particular system was chosen because at this location *P. radiata* grows throughout the year^{1,2}. Wide rings (2 cm) are laid down which enables different parts of the ring, and therefore wood laid down at different temperatures, to be sampled.

The isotopic composition of the atmospheric precipitation varies only slightly between winter and summer (about 20‰) in deuterium content³ so that any temperature effects are not masked by changing isotopic composition of the atmospheric precipitation during the year. During the year in Hamilton both the monthly mean maximum daily temperature and the monthly mean daily temperature vary through a cycle with an amplitude of 10°C . Thus the method enables even small temperature-induced isotopic effects to be detected.

In Fig. 1 we present a plot of the deuterium/hydrogen (D/H) ratio of the C-H hydrogens of cellulose across two rings laid down during the period 1919–20 by a *P. radiata* tree growing in Hamilton, New Zealand. Cellulose was prepared by the standard techniques of wood chemistry (see for example ref. 4). Before the cellulose was combusted the exchangeable hydrogens on the hydroxyl groups were brought to a standard D/H by equilibration with water of known D/H (7 d, 95°C). The results are reported in per mille deviation from standard mean ocean water⁵. The system is at least 30‰ depleted in the summer as compared with the winter and seems to record the annual temperature cycle. It should be noted that the isotopic composition of the cellulose varies in the opposite direction to the isotopic composition of the atmospheric precipitation, which is more depleted in deuterium in the winter and less depleted in the summer. Thus the effect could not be the result of the changing of the isotopic composition of the precipitation. Similarly the results could not reflect changes in the isotopic composition of cell sap since cell sap would, if anything,

Fig. 1 Variation in isotopic ratio of the C-H hydrogens in cellulose across tree rings.



become less depleted in deuterium in summer due to greater evapotranspiration associated with higher temperatures and lower humidity. It is therefore concluded that the D/H ratio of cellulose in the wood of *P. radiata* changes with temperature due to the temperature effects on one or more of the biochemical reactions leading to cellulose. Taking into account the isotopic variations of the atmospheric precipitation we obtain a temperature coefficient for the C-H hydrogens of cellulose of -5% per $^{\circ}\text{C}$.

Most probably this change reflects the air temperature since it is the biochemical pathways leading to the synthesis of the sucrose in the leaves which lead to the formation of the C-H bonds. The sucrose is translocated to the trunk where it is converted to cellulose. If it is indeed a temperature effect on the biochemical steps leading to the synthesis of sucrose, then the effect described in this communication should be found to occur generally in C-3 plants. The temperature coefficient of this biochemical thermometer could enable past temperature measurements to be measured to better than 0.1°C which should be more than adequate for studying past climate changes.

The other isotopes both in cellulose and in the other compounds in wood are being studied and will be reported elsewhere.

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Stability of Lotka-Volterra systems

NONLINEAR systems are very often studied in terms of simple mathematical models. The Lotka-Volterra equations provide such a model and have been used to study physical, chemical, ecological and social systems¹. An important question in the analysis of these equations is the stability of the equilibrium values of the interacting variables. Most work with Lotka-Volterra models has considered only neighbourhood stability against small perturbations away from equilibrium. The question of global stability when large perturbations from equilibrium are involved has only been analysed in a few special cases². We establish here that for the general Lotka-Volterra models local stability ensures global stability (asymptotic stability in the large). This result provides justification for the work that has been and is being done^{2,3} on linearised versions of the Lotka-Volterra models.

Our emphasis here will be on ecological systems: we consider a set of interacting biological species, but that does not restrict the analysis. If N_i is the biomass (or population) of species i , it changes with time t according to the generalised Lotka-Volterra equations

$$dN_i/dt = N_i \left(b_i + \sum_j a_{ij} N_j \right)$$

Here b_i are growth rates in the absence of interactions, and a_{ii} and a_{ij} ($i \neq j$) are intraspecies and interspecies interaction coefficients, respectively. We make no assumption about the

elements a_{ij} , except that they are real. Equilibrium values of the biomass, N_i^* , are defined by $dN_i/dt = 0$, or

$$b_i = - \sum_j a_{ij} N_j^*$$

We consider 'feasible' models, where all $N_i^* > 0$.

A local stability analysis is carried out by introducing small perturbations $y_i = (N_i - N_i^*)$, and obtaining a linear system $dy/dt = Ay$. Here y is the vector of y_i , and the 'community matrix' A has elements $(a_{ij} N_j^*)$. This system is stable, or equivalently the matrix A is stable, if all of the eigenvalues of A have negative real parts. The stability of A guarantees the local stability of the system about the equilibrium values N_i^* .

To examine global stability, we first introduce variables x_i through the substitution $N_i = N_i^* \exp(x_i)$. The x_i then obey the equation

$$dx_i/dt = \sum_j a_{ij} N_j^* [\exp(x_j) - 1]$$

Let x be the vector of x_i . Consider the function⁴

$$V(x) = \sum_i [\exp(x_i) - x_i - 1]$$

Observe that $V(x)$ is positive definite, continuously differentiable, and that $V(x) \rightarrow \infty$ as $|x| \rightarrow \infty$. The time derivative is

$$dV(x)/dt = \sum_{i,j} [\exp(x_i) - 1] a_{ij} N_j^* [\exp(x_j) - 1]$$

Now, if the derivative dV/dt is negative definite, the function $V(x)$ would be a Lyapunov function for the system, and would ensure global stability about the equilibria N_i^* .

The conventional way of writing quadratic forms is in terms of a symmetric matrix. So we define a symmetric matrix $B = (A + A')/2$, where the prime indicates the transposed matrix. Then we rewrite dV/dt as a quadratic form with the elements of B , b_{ij} , as

$$dV/dt = \sum_{i,j} [\exp(x_i) - 1] b_{ij} [\exp(x_j) - 1]$$

Now if B is a negative definite matrix, dV/dt is negative definite, and V is a Lyapunov function.

The theorem of Lyapunov⁵, states that a matrix A is stable (in the sense that the real parts of its eigenvalues are all negative), if, and only if, there exists a positive definite matrix C such that $(CA + A'C)$ is negative definite. We choose $C = I/2$ where I is the unit matrix. With this choice, our earlier matrices A and B are related by $B = (CA + A'C)$. Thus the theorem states that local stability (stability of our matrix A) occurs if, and only if, B is negative definite. And the negative definiteness of B makes $V(x)$ a Lyapunov function, ensuring global stability.

Thus for the general Lotka-Volterra systems, local stability implies global stability, and vice versa. This result is a happy consequence of the general form $dN_i/dt = N_i F_i(N)$ and the linearity of the F_i . This result does not apply to situations where the 'community matrix' A is critical, that is, has eigenvalues with vanishing real parts. The special cases mentioned earlier for which global stability has been studied follow from the insertion of specific assumptions about the interaction coefficients. The implications for any specific system must of course be considered within the definite limitations of the Lotka-Volterra model as a representation of reality.

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Recovery process of the sensitive plant

THERE is a long history of study of the unusual behaviour of the 'sensitive plant', *Mimosa pudica* L., stretching back to the *Enquiries into Plants*, written by Theophrastus in about 300 BC. A large literature has built up, much of which can be traced from the papers of Pickard¹, Sinyukhin and Gorchakov², and Weintraub³. In spite of this, very little work seems to have been done on the detailed kinematics of the recovery of the plant following a collapse. We report here the results of a study of the recovery of the primary petiole after seismonastic stimulation, that is, after collapse induced by a blow.

Plants used in this study were all grown from seed obtained from commercial seedhouses. The temperature was kept in the range $21 \pm 3^\circ\text{C}$ by suitable thermostatic controls. The lighting was supplied exclusively by a battery of eight 35-W fluorescent tubes mounted horizontally 24 cm above the plant pots. The sequence used was about 12 h light followed by 12 h darkness, the dark period being from 1700 to 0500. Plants were investigated at various ages in the range 1–18 months. They were grown in John Innes number 2 mixture, germinated in closed polythene containers and then transplanted into 2-inch pots.

Preliminary films of the collapse and recovery of plants taken 'end-on' to various primary petioles showed that the motions of the latter were always planar, although the motions of the secondary pinnae were more complex and sometimes gave an illusion of non-planar motion of the primary petiole. It was therefore convenient to place the plant in front of a vertical screen covered with 1-mm graph paper and orientate it so that the plane of motion of the primary petiole on which attention was to be focused was roughly parallel to the screen. A Sankyo CME 660 Super-8 cine camera was mounted so that its optical axis was roughly normal to the screen and passed close to the primary pulvinus connecting the stem of the plant to the primary petiole of interest. Preliminary experiments showed that the collapse takes a couple of seconds, whereas the recovery can take an hour or more. The camera was therefore run at its normal speed of 18 frames s^{-1} while the plant was being stimulated and collapsing, but thereafter only one frame was used every 5 s to obtain time-lapsed film of the recovery. As a check on the timing a stopwatch calibrated in 0.1-s intervals was mounted near the plant and filmed with it.

Since in most cases the primary petiole is approximately straight at all times the angle θ which it makes with the horizontal provides a quantitative measure of its geometrical condition. The graph paper on the screen was of assistance in measuring this angle. Any error in its measurement due to the direction of observation not being quite normal to the plane of motion would be small and would not affect either the general form of the θ -time curves or the periods of any oscillations contained in these.

Detailed observations were made of numerous collapses and recoveries using the plants at various ages and at a variety of times of day. The collapse process, which was always complete within 2–3 s, showed no particularly surprising features (detailed quantitative study will be reported elsewhere). The recovery process on the other hand, which usually took about an hour for completion, showed some rather unexpected features. The curve of θ against time for the recovery could take any one of three different forms which showed strong similarities to the displacement-time curves for an unforced damped harmonic oscillator. The first two forms (Fig. 1a and b) are similar to the two possible types of behaviour of a heavily damped harmonic oscillator, and accounted for 10 and 30%

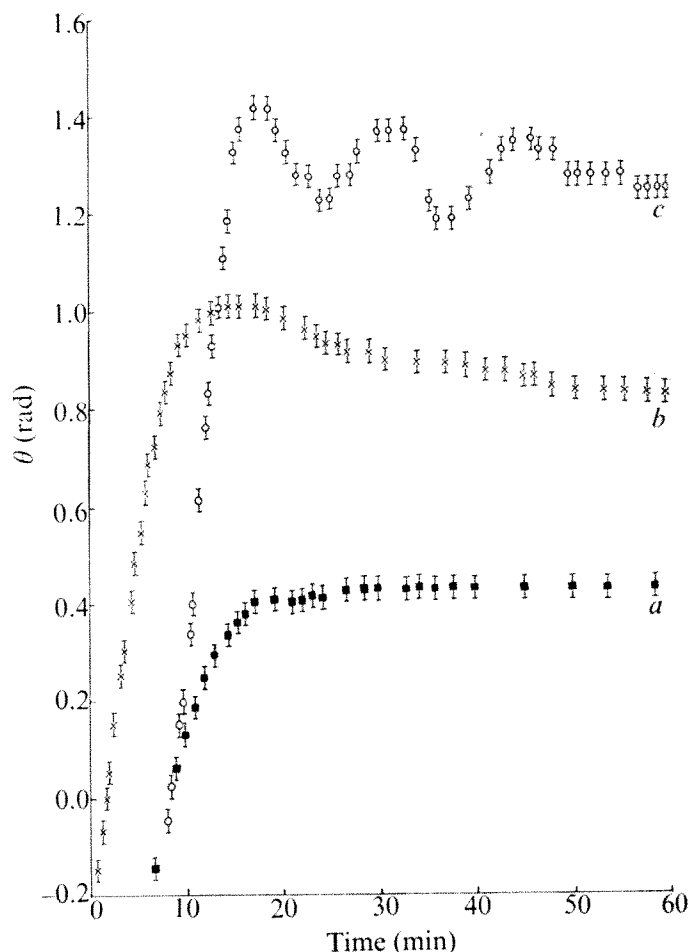


Fig. 1 Examples of the three types of recovery: a, heavily damped mode (undershooting type); b, heavily damped mode (overshooting type); c, lightly damped mode.

of the observed recoveries, respectively—referred to jointly as the heavily damped mode. The third form (Fig. 1c) is similar to the lightly damped harmonic oscillator curve. The period of oscillation was typically about 15 min. It accounted for the remaining 60% of observed recoveries. This similarity to the damped harmonic oscillator does not extend to the fine detail. In the lightly damped mode, for example, the successive displacement maxima and minima do not obey the log. dec. rule. In view of the complex internal structure of the primary pulvinus it is not surprising that the response should deviate somewhat from that of a damped harmonic oscillator: the surprise is that it should show any resemblance to it at all. In fact it seems quite likely that the system can be modelled by the usual spring-mass-dashpot system with some simple nonlinearities imposed—for example, anisotropic damping.

In addition to the main observation that a primary petiole behaves in a manner resembling a damped harmonic oscillator a number of other interesting facts emerged from our study. First, different primary petioles of the same plant can recover by way of different modes at the same time. Second, a given petiole at a given age always recovers by way of the same mode. If this mode happens to be the heavily damped one, however, its type may change. Third, on the first stimulation after a long rest period (say, at least 24 h) the final equilibrium position reached differs from that before stimulation, but immediate restimulation produces no further change in the equilibrium position. Finally, for a given petiole recovering by way of the lightly damped mode the period of oscillation is greater on second and subsequent stimulations than on the first after a long rest period. In a typical case we found that the initial period was about 15 min but that this increased by about 30%

on the second stimulation with little further change on the third.

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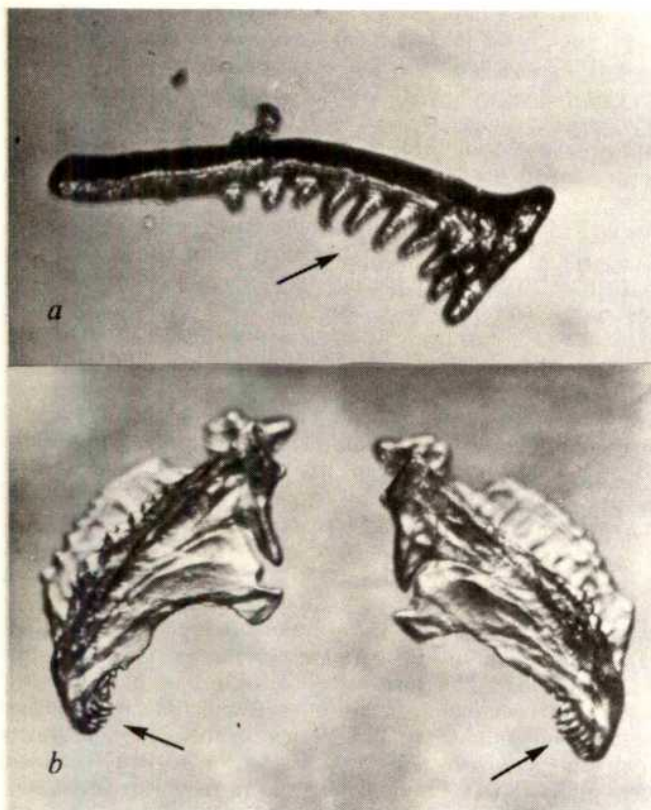
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Odontoid processes in pipefish jaws

ABSENCE of jaw teeth of any kind has been considered a familial character in pipefishes (Syngnathidae)^{1–3}, and their feeding mode has generally been described as a picking or sucking process resulting from rapid intake of water through the elongate snout^{4,5}. During systematic studies of Indo-Pacific syngnathids, we have found toothlike processes on the premaxillae and dentaries in three genera of abdominal-pouch pipefishes (Gastrophori). These structures (Fig. 1) are best developed in *Choeroichthys sculptus* (Günther) and *C. brachysoma* (Bleeker) where they are readily seen under $\times 30$ magnification in all subadults and adults. In *Syngnathoides* Bleeker (dentaries only) and a newly described genus⁶, they are inconspicuous, and best seen in cleared and alizarin-stained material.

Histological sections (from *Choeroichthys sculptus*) show no evidence of enamel, pulp cavity or basal differentiation in these processes and they appear to be odontoid projections of bone, rather than true teeth. Nevertheless, location and gross morphology suggest that they serve as functional teeth, and that they may be used in browsing or some other previously unreported mode of pipefish feeding. Data on food items are unavailable, but the habitats of these forms lend some support to this

Fig. 1 Odontoid processes of *Choeroichthys sculptus*. a, Right premaxilla, maximum length about 0.8 mm; b, mandibles, maximum length about 0.9 mm. Arrows indicate 'teeth'.



assumption. All *Choeroichthys* species and the undescribed genus are associated with coral or rocky bottoms; *Syngnathoides*, monotypic, is reported to live on weeds³. Each of these habitats would provide suitable niches for grazing pipefishes.

These odontoid processes constitute a newly recognised character of systematic importance and may prove significant in studies of pipefish phylogeny.

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Corpus allatum and ovarian growth in a polymorphic paedogenetic insect

IN parthenogenetically reproducing *Aphis craccivora* Koch, ovarian growth is initiated before birth and continues throughout larval life¹. Consequently, maintenance of the juvenile form and reproduction occur simultaneously in this insect. The corpus allatum has been shown to be active in both these processes in other insects where ovarian growth does not commence until after the larval stages are completed². To elucidate the role of the corpus allatum in the two simultaneous processes in *A. craccivora*, I have studied the gland and ovarian development during the larval stage. Both apterous and alate aphids were used as these two morphs have a different ovarian growth pattern in *A. craccivora*¹.

Aphid ovaries were examined from birth to the onset of larviposition by dissection in distilled water after rapid fixation in Gilson's fluid. An index of ovarian development was found by calculating the total length of oocytes and embryos present in the body. Aphid heads were fixed in Gilson's fluid, sectioned sagittally at 6 μ m and stained with Ehrlich's haematoxylin. Corpus allatum nuclei were measured with an eyepiece micrometer and the mean nuclear size was calculated using the mean product of the long and short diameters of four nuclei in the gland³.

The mean nuclear size of the corpus allatum was positively correlated with the ovarian index in both apterae ($r = 0.91$, $P < 0.05$) and alatae ($r = 0.95$, $P < 0.05$) up to the onset of larviposition. This correlation was maintained in each morph even though their ovarian development is out of phase. Apterous have a greater corpus allatum mean nuclear size and ovarian index than alatae during larval life until the adult moult when larviposition occurs in apterae. But the two parameters continue to increase in alatae until larviposition occurs approximately 2 d after the adult moult.

The growth of the corpus allatum during the larval stage was enormous compared with that of body length and head width. Corpus allatum volume increased elevenfold from birth to the final moult while body length and head width showed a fourfold increase. The mean length of the terminal ovarian follicle increased tenfold in the same period.

My results provide strong evidence that the corpus allatum in *A. craccivora* is simultaneously involved in ovarian growth and maintenance of the juvenile form during the larval stage. Further proof for the extra role of the corpus allatum during larval life in *A. craccivora* compared with non-paedogenetic insects is given by the measurements of relative growth of the gland and other body parts. A comparative study of several

holometabolous and hemimetabolous insects² showed little growth of the corpus allatum during the larval stage compared with the increase in body length and head width—the opposite result to that obtained with the paedogenetic *A. craccivora*. As ovarian growth and maintenance of the juvenile state are simultaneous processes in this aphid it is likely that only one corpus allatum hormone is involved in both processes.

The comparison of apterous and alate aphids correlates well with previous studies on the role of the corpus allatum in wing polymorphism⁴ and the total involvement of the gland in aphid physiology is now more apparent. The higher corpus allatum activity in apterae during the larval stage promotes faster ovarian growth and preserves the juvenile form, while in alatae, the development of wing buds and reduced ovarian growth are both coincident with a lower corpus allatum activity.

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Silent allele as genetic basis of fucosidosis

SEVERAL individuals have been recognised as having an almost complete deficiency of the lysosomal hydrolase α -L-fucosidase (EC 3.2.1.51) in their tissues and body fluids¹⁻⁷. This condition (designated fucosidosis) is associated with the accumulation of fucose-containing glycolipids and glycoproteins in various tissues¹⁻⁵. The clinical manifestations include coarse facial features, progressive psychomotor regression, susceptibility to respiratory infections and severe neurological signs, including generalised spasticity. Two distinct forms of fucosidosis (types 1 and 2) have been recognised⁷. Both are characterised by a virtually complete deficiency of α -fucosidase activity when assayed with artificial substrates. Physical and mental deterioration, however, proceeds far more rapidly in type 1 and these patients do not survive beyond early childhood. Type 2 patients can survive to adult life³. Additional features which distinguish the two forms of the disease are the presence, in type 2 only, of the skin lesion, angiokeratoma corporis diffusum, and an elevation in type 1 only, of the NaCl concentration in sweat⁷.

We have described a common polymorphism of α -fucosidase in the North American population⁸. Using isoelectric focusing in acrylamide gel and a specific staining technique we were able to distinguish three distinct phenotypes in neuraminidase-treated leukocyte extracts from different normal individuals (Fig. 1). Treatment with neuraminidase converted the six or more isozymes present in the original extract to one of the relatively simple patterns shown in Fig. 1, apparently by removing sialic acid residues from the more acidic isozymes and thus converting them into the less acidic forms⁹. Family studies showed that the three common phenotypes were attributable to the existence of two codominant alleles at a single, autosomal gene locus. These alleles were designated Fu^1 and Fu^2 . Thus, individuals of phenotypes Fu^1 , Fu^2 and Fu^{2-1} had the genotypes Fu^1Fu^1 , Fu^2Fu^2 and Fu^2Fu^1 , respectively.

We have studied the segregation of these two common alleles at the α -fucosidase gene locus in the family of two brothers affected with fucosidosis 2. The results have thrown some light on the genetic basis of the disease. The pedigree of the patients' family is shown in Fig. 2. The three phenotypes Fu^1 , Fu^{2-1} and Fu^2 were all observed in leukocytes from family members. None of the α -fucosidase isozymes was detected in leukocytes, cultured long term lymphoid lines, fibroblasts or serum from the two affected children. With this exception,



Fig. 1 Isozyme composition of the three common α -fucosidase phenotypes after isoelectric focusing of neuraminidase-treated leukocyte extracts in acrylamide gel⁸. *a*, Part of an acrylamide gel stained with 4-methylumbelliferyl- α -L-fucoside and photographed in ultraviolet light. Four samples shown have the phenotypes (from left to right) Fu^1 , Fu^{2-1} , Fu^{2-1} and Fu^2 . *b*, Diagrammatic representation of the three common phenotypes. Arrows show the approximate pH range within which the various isozymes focused.

isozyme patterns among family members did not differ from those seen in the general population. There exist within this family, however, two patterns of inheritance of α -fucosidase phenotypes which are clearly not consistent with the simple segregation of two codominant alleles. The first incompatibility is between the paternal grandmother (I5) of the two affected children and her daughter (II4), with the mother having the Fu^2 phenotype and the daughter the Fu^1 phenotype. A photograph of the isozymes seen in leukocytes from these individuals is shown in Fig. 3. In the normal course of events the daughter would inevitably have received an Fu^2 allele from her mother and would therefore have an Fu^2 or Fu^{2-1} phenotype, depending on whether she received an Fu^1 or Fu^2 allele from her father.

If one rules out such unlikely events as fresh mutation or non-biological parenthood (particularly unlikely as it is mother and daughter who are incompatible) then the presence of a silent (or 'null') allele at the α -fucosidase locus is the only satisfactory explanation for the observed segregation of phenotypes. This allele, which we will call Fu^0 , is silent in the sense that its product (if any) is not detectable by the assay methods used in this study. Having introduced the Fu^0 allele the explanation for the observed pattern of inheritance becomes straightforward. The paternal grandmother (I5) has the genotype Fu^2Fu^0 and consequently an Fu^2 phenotype. Her daughter (II4) received an Fu^0 allele from her mother and a normal Fu^1 allele from her father, resulting in the genotype Fu^1Fu^0 and an Fu^1 phenotype (Fig. 2).

The second example of apparent incompatibility between parent and child is between I2, who has an Fu^2 phenotype, and his son (III) who is Fu^1 . This situation can also be resolved by proposing that a silent allele is present. In this case the father must have the genotype Fu^2Fu^0 , resulting in an Fu^2 phenotype, whereas the son has the genotype Fu^1Fu^0 , having received a normal Fu^1 allele from his mother (II) and an Fu^0 allele from his father.

Because of the unusual inheritance of α -fucosidase phenotypes within this family we examined the segregation of phosphoglucomutase (locus 1), esterase D and red cell acid phosphatase phenotypes. All these enzymes are coded by two or

more common alleles in human populations¹⁰⁻¹². Throughout the family the inheritance of these polymorphic enzymes was completely normal and consistent with the relationships shown in Fig. 2.

If a silent allele is segregating at the α -fucosidase locus within this family it is likely that the two children affected with fucosidosis and with virtual absence of α -fucosidase activity, are in fact homozygous for this allele. If this is so then their parents must be carriers of Fu^0 and must therefore have either an Fu^1 or Fu^2 phenotype. An Fu^2 -1 phenotype is clearly impossible. As shown in Fig. 3, the two parents (II5 and II6) have the phenotypes Fu^2 and Fu^1 , respectively, presumably with the genotypes Fu^2Fu^0 and Fu^1Fu^0 .

Based on the assumptions that the three alleles Fu^1 , Fu^2 and Fu^0 are segregating within this family and that individuals marrying into the family have two normal alleles, we have been able to derive unambiguous genotypes for most of its members. In three cases, however, it was not possible to do this on the basis of phenotyping alone. Individuals I3, II8 and III3, all of whom have the Fu^1 phenotype, could be of genotypes Fu^1Fu^1 or Fu^1Fu^0 , with equal probability. In these cases the genotypes shown in the pedigree were assigned on the basis of the α -fucosidase activity of peripheral leukocytes, on the assumption that individuals carrying the silent allele should have a lower level of activity than individuals with two normal alleles. A comparison of obligatory carriers of Fu^0 (that is, I2,5,7 and II1,4,5,6) with normals (that is, II1,4,6 and II2,3,9) supports this assumption. Average α -fucosidase activity in obligatory carriers of Fu^0 was 132 ± 27 nmol per h per mg protein, whereas in normals the equivalent value was 243 ± 59 . Leukocyte activities for individual members of the family are shown in Fig. 2. The present results support previous findings which showed that a high proportion of carriers for fucosidosis (that is, heterozygotes for Fu^0) could be detected by enzyme assay methods¹³.

In each branch of the family the Fu^0 allele must have been passed down by way of the grandmother (I5 and I7) as both grandfathers are Fu^2 -1 heterozygotes and must therefore have two normal alleles. Note that whereas the two grandfathers are of Polish and German descent, the two grandmothers are both of Southern Italian parentage. Of six other families reported with fucosidosis¹⁻⁶ (four with type 1 and two with less severe type 2), three have been of Southern Italian origin (two type 1 and one type 2).

In the family described, the presence of a silent allele at the

Fig. 2 Pedigree showing segregation of common alleles Fu^1 and Fu^2 and the silent allele Fu^0 in the family of two sibs with α -fucosidase deficiency. Numbers 1, 2 and 2-1 are α -fucosidase phenotypes determined by isoelectric focusing. Leukocyte α -fucosidase activities (nmol 4-methylumbelliferone released per h per mg protein) are shown in parentheses. Two affected children are indicated by arrows. A clinical description of these two children has been presented elsewhere⁷.

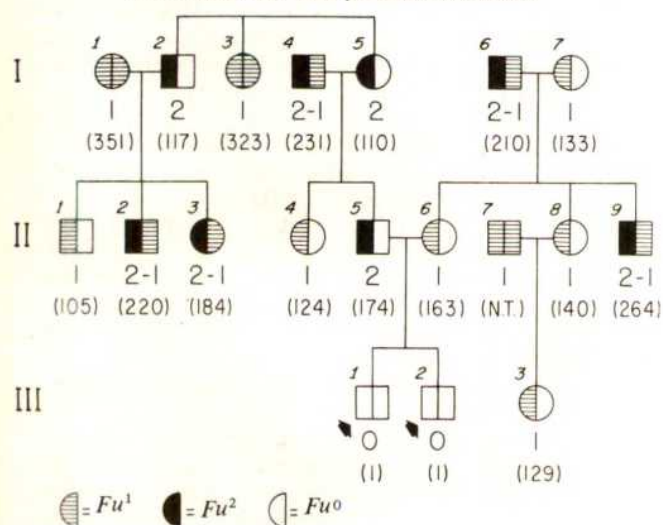


Fig. 3 Isozyme composition of α -fucosidase in leukocyte extracts from some members of the family shown in Fig. 2. Pedigree number of each individual is shown. α -Fucosidase phenotypes (from left to right) are Fu^2 -1, 2, 2-1, 1, 1, 2 and 1.

α -fucosidase locus has been deduced from apparent anomalies in the inheritance of two normal alleles at that locus. Although mutations at loci coding proteins required for activation, stabilisation or regulation of α -fucosidase could certainly account for a reduction in enzyme activity in the heterozygous state and a deficiency in homozygotes, such mutations cannot explain the qualitative abnormalities in the inheritance of the three common α -fucosidase phenotypes described here. On the other hand, a mutation within the structural locus for α -fucosidase, resulting in an inactive or silent allele at that locus, provides a satisfactory explanation for both the qualitative and quantitative aspects of α -fucosidase inheritance within this family.

Rare silent alleles have been detected during studies on the inheritance of polymorphic proteins in normal families¹⁴. Only recently, however, have family studies of this type been used to implicate structural gene mutations as a cause of human disease^{15,16}. As it has been estimated¹⁷ that 25-30% of all human enzymes have common polymorphisms detectable by electrophoresis alone, studies of this type should be widely applicable to the study of inherited enzyme deficiencies.

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Phagocytosis and cytotoxicity by a macrophage tumour and its cloned cell line

MACROPHAGES found in lymphoid and other tissues are defined by adherence, staining, morphology and rapid pinocytosis. Subtypes of macrophage-like cells have been distinguished on the basis of density and functional properties¹⁻³. Immune macrophages⁴ and normal macrophages in the presence of specific antiserum⁵⁻⁸ will lyse relevant target cells, and can phagocytose cells by an independent mechanism⁹⁻¹², but it has not been shown that the same cell type can carry out both functions.

We have described a murine reticulum cell sarcoma (J774), whose ascites form has the macrophage properties of adherence, morphology, receptors for immunoglobulin, and antibody-dependent lysis of target cells¹³. The tumour-cell pellet contains 10–50 µg lysozyme per 10⁷ cells, and as much as 200 µg ml⁻¹ of the enzyme is found in the ascitic fluid. This tumour has now been adapted to culture and cloned. Both the cell line and ascites cells exhibit phagocytosis and lysis of target cells, dependent on specific antibody, but the cell line is mainly phagocytic, and the fresh tumour is mainly cytotoxic. The result is influenced greatly by the method of assay, and the effector cell population can be rapidly switched from one mode of attack to the other. Cultured cells produce typical ascites when reintroduced into mice, but require several days *in vivo* to change from high levels of phagocytosis to cytotoxicity.

J774 ascites cells are highly cytotoxic in the presence of specific antiserum lysing 50% of target red blood cells (RBCs) in 4 h at an effector target cell ratio of 2 : 1 (Fig. 1a). Low but significant lysis is seen even at a ratio of 1 effector cell to 10 RBCs. We have shown¹³ that the small number of host peritoneal cells contained in the ascites fluid are not responsible for this killing effect. Only a small proportion of cells are phagocytosed in the same assay using tissue culture dishes. When the assay is performed in plastic tubes, the effector cells exhibit less cytotoxicity and much more phagocytosis, also dependent on specific antiserum. Wide tubes used to simulate the shallow fluid depth in culture dishes give results similar to narrow tubes. Tumour cells adhere more strongly to the dishes than tubes, and this may be a signal favouring cytotoxicity.

J774 ascites cells put into culture did not grow initially, and their cytotoxic activity gradually declined over several months. A cell line was derived after one passage back into mice, which grows partly adherent and partly floating, with a doubling time around 24 h. When subcultured separately, adherent and non-adherent cells will each generate the mixed cell population. The culture cell line also synthesises large amounts of lysozyme.

The cell line exhibits minor cytotoxicity but predominantly antibody-dependent phagocytosis (Fig. 1b). The adherent and non-adherent cells tested separately give identical results. The two culture types may represent different stages of the cell growth cycle, or a response to culture conditions, such as limited surface area, although the culture dish is not visibly crowded when floating cells appear. Most of the lysis or ingestion of RBCs occurs in the first 2 h of incubation, and radioactivity within the macrophage tumour cells is retained for more than 4 h thereafter. This culture line has been reinjected into mice many times, and the resulting ascites cells always show the strong antibody-dependent cytotoxic activity of the original tumour line (Fig. 1c).

The two effector mechanisms may be carried out by two different cell lineages coexisting in the ascites and culture populations. The growth conditions could thus influence which cell type and effector function dominated. Therefore, the culture line was cloned and tested. The cloned culture J774.1 mainly phagocytoses cells rather than lysing them (Fig. 1d), similar to the parent culture, and the ascites form of the cloned cell line mainly kills targets (Fig. 1e). Normal spleen cells, at

50-fold higher concentrations, have the cytotoxic capacity of ascites preparations, but exhibit no phagocytosis (Fig. 1f).

How rapidly can the effector cell population change its handling of antibody-antigen conjugates? Most of the killing or ingestion of RBCs by J774 occurs in the first 2 h either in the tube or dish assay using 0.5 RBCs per J774. J774 ascites cells were therefore incubated for 2 h at 37 °C in tubes which favoured phagocytosis, and then washed and tested for reaction with labelled RBCs in dishes. Most of the macrophage cells were recovered by vigorous pipetting. Preincubation alone in tubes has no effect on the subsequent ability of J774 to kill targets in dishes (90% of control killing shown by cells kept on ice during preincubation). With antiserum and 2 RBCs per J774, the subsequent killing activity was 70% of that for controls. When preincubated with antiserum and 10 RBCs per J774, during which 50% RBCs are phagocytosed with 95% of the macrophage cells containing at least one erythrocyte, the subsequent killing of newly added targets with antiserum was only 25% of that for controls. This high dose inhibition may be due to competition for macrophage surface receptor sites by antigen-antibody complexes, as well as to physiological changes resulting from massive phagocytosis.

Thus, the population of J774 effector cells can be rapidly and reversibly switched between two different mechanisms of handling target RBCs. But, the culture line still does not show the very high killing activity of the ascites preparation. To study this transition, we passaged culture cells into mice intraperitoneally and tested for antibody-dependent cytotoxicity during the next few days (Table 1). One day after *in vivo* passage, cells still resembled fresh cultured cells, whereas ascites passaged 1 d

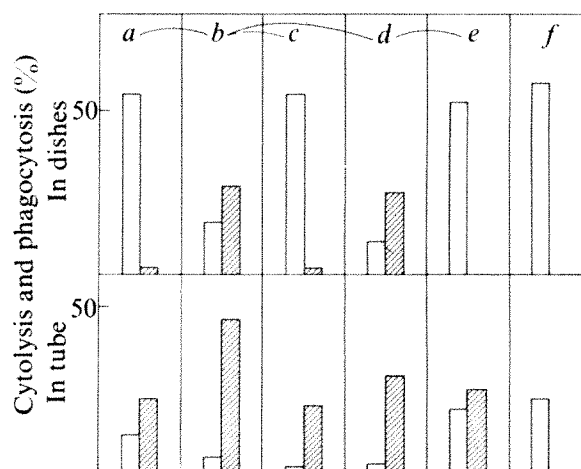


Fig. 1 Cytotoxicity (open columns) and phagocytosis (hatched columns) by J774 effector populations. Assays were performed according to Walker and Demus¹² using 2×10^5 J774 cells or 10^7 spleen cells, 10^5 ⁵¹Cr-labelled sheep RBCs and 10 µl of 1:10 mouse anti-RBCs or 1:10 normal mouse serum¹³ in 1 ml volumes containing 5% heat inactivated foetal calf serum. Cultures were incubated 4 h in rocking tissue culture dishes (Falcon 3001) or stationary tubes (Falcon 2038). Plastic Petri dishes (Falcon 1008) gave less killing and phagocytosis, and large tubes (Falcon 2051), which more nearly duplicates the large area and short liquid depth of dishes, gave results identical to the small tubes. Very little effector activity was seen in assays using glass tubes. Antibody-dependent target lysis was determined by release of ⁵¹Cr to the medium, and phagocytosis by water-lysis resistant radioactivity in cell pellets corrected¹³ for control values with normal serum (about 7% lysis and 14% water-resistant counts). These control values were similar to those from incubations without mouse serum or in which effector cells were replaced by 10^7 chicken RBCs. Each result is the average of several experiments using duplicates, with standard errors $\leq 7\%$ of means. a, c and e, Ascites cells collected from mice at 10–20 d after intraperitoneal inoculation with 0.1 ml ascites fluid (a) or 10^7 culture cells (c and e). b and d, J774 cells growing in Eagle's medium containing 10% foetal calf serum, after more than 100 doublings of the population *in vitro*. The cells in d were cloned by limit dilution in a row of 8 wells inoculated with approximately 2 cells per well, only one well developing a colony of cells, which appeared as a single focus. f, Normal spleen.

Table 1 Transition from phagocytosis by culture cells to lysis by ascites cells

Cells	Days <i>in vivo</i>	Effector activity			
		Dish		Tube	
		Lysis	Phagocytosis	Lysis	Phagocytosis
Culture	0	6	31	0	48
	1	11	35	0	61
	3	15	21	2	55
	10-20	54	3	11	25
Ascites	1	41	13	0	31
	10-20	55	11	6	29

Effector cell activity was assayed as in Fig. 1. J774 culture cells were used directly (day 0), or 1-20 d after transfer to mice intraperitoneally. Ascites cells were used 1 d or 10-20 d after transfer to mice intraperitoneally.

in mice showed the typical ascites response. Three days after *in vivo* passage, the culture cells showed effector levels intermediate between the culture line and long term ascites. Ascites resulting from culture cells passaged 10-20 d in mice behave as typical ascites preparations.

We believe these experiments demonstrate that a single cell lineage of macrophages can both phagocytose cells without immediate release of labelled components and directly lyse antibody-coated target cells. The evidence includes: first, the unlikelihood of two different macrophage tumours originating in the same mouse (we have seen only two reticulum cell sarcomas among more than 500 tumours during a myeloma induction programme in which J774 occurred); second, the unlikelihood of two tumours coexisting through many animal and culture passages; third, the reversible shift from lysis by ascites preparations to phagocytosis by culture cells; and finally, identical results with a cloned cell line. The assay conditions largely determine which mechanism predominates. It is not clear, however, if the same cell can carry out both functions rapidly at the same time. The effector populations studied may be composed of subgroups at different stages of differentiation. The slow transition over several days from the phagocytosis exhibited by cells grown in culture to the predominant cytotoxicity of ascites cells suggests that changes in the total population occur after one or more rounds of cell division in *in vivo* or *in vitro* conditions. It will be interesting to see if normal macrophages stimulated by growth factors to divide in culture¹⁴ can be switched from cytolysis to increased phagocytosis. An SV40-transformed macrophage cell line described by Walker and Demus¹² *in vitro* shows a high level of phagocytosis similar to cultured J774. Another macrophage-like cell line derived from a methylcholanthrene-induced 'lymphoid' neoplasm has been described by Koren, Handwerger and Wunderlich¹⁵ as being highly active in killing target cells, although a comparison of target phagocytosis was not done.

The ability to manipulate a cloned line of cells with several effector functions will facilitate further understanding of the role of macrophages in immunity.

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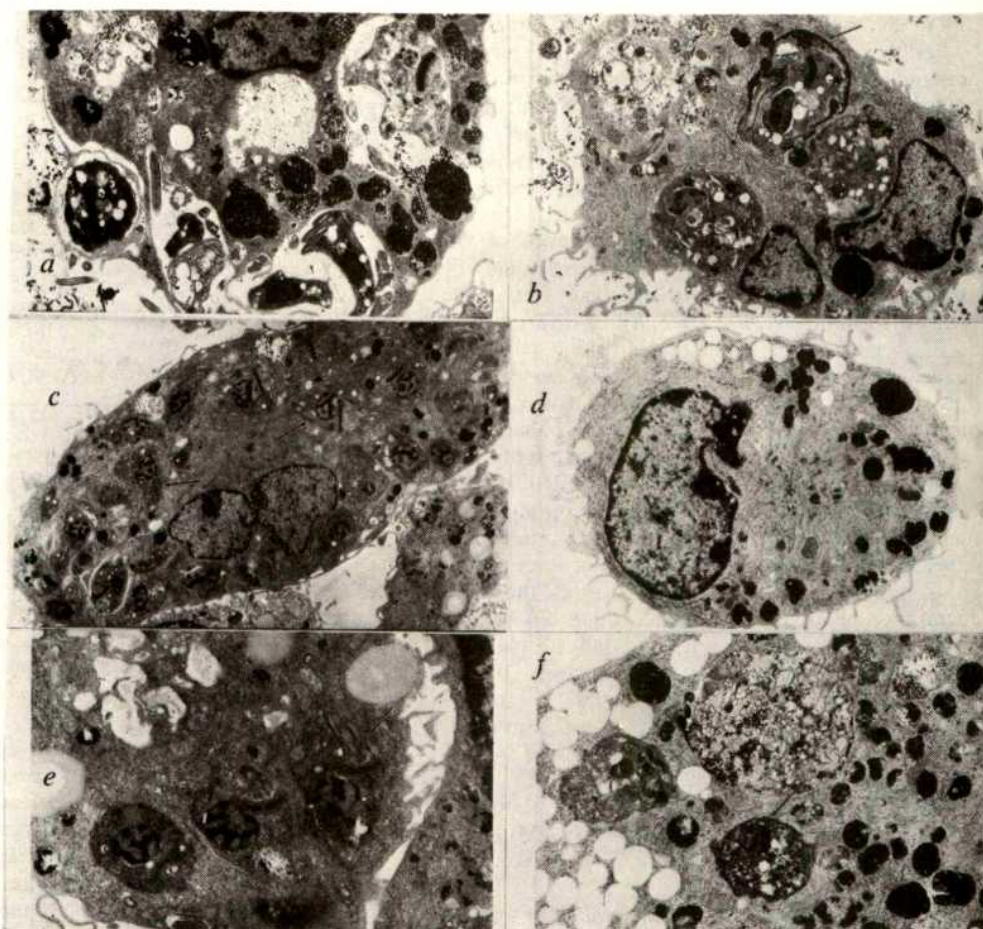
Resistance of *Trypanosoma cruzi* to killing by macrophages

CHAGAS' disease is a chronic debilitating illness, widespread in Latin America, caused by infection with the haemoflagellate, *Trypanosoma cruzi*. Antibodies to the organism, commonly present in both the acute and chronic forms of the disease, have generally been ineffective in protecting animals against infection^{1,2}. There is ample pathological documentation of the involvement of macrophages in Chagas' disease³⁻⁵ but unfortunately little is known about their function, or of the role of cell-mediated immunity in general. It has, however, been shown that passive transfer of immune spleen cells can protect mice against *T. cruzi* infection¹. Because macrophages serve as effector cells in protecting against intracellular parasites, we have studied the interaction of *T. cruzi* and macrophages *in vitro*. Preliminary experiments showed that at low parasite-macrophage ratios (for example 1) most cultures of normal uninduced mouse peritoneal macrophages could eliminate the infection and survive; in contrast, with higher ratios (for example 10), they were destroyed⁶. Mackaness⁷ has shown that macrophages can be 'activated' after immunisation and challenge with specific antigen to become non-specifically resistant to other infecting bacteria. We report here that nonspecifically 'activated' macrophages have heightened resistance to intracellular growth of *T. cruzi*, and suggest that its absence in normal macrophages is related to the ability of the parasite to escape from the phagolysosomes into the cytoplasm.

The Tulahuen and Ernestina strains of *T. cruzi* were supplied by Dr Franklin Neva of the National Institutes of Health, and cultured as described previously⁸. Normal macrophages were collected from the peritoneal cavity of C57BL/10.D2 mice by lavage with ice-cold Hanks' solution containing 5 IU ml⁻¹ of heparin. Macrophages which we term activated were obtained by immunising mice with living BCG (5 × 10⁶ Phipps strain, intraperitoneally) and challenging the mice 21 d later with 10⁷ BCG given intraperitoneally. The peritoneal macrophages were collected 3 d later.

The term activated macrophages has been used in the past to denote different, and not necessarily related properties, for example more rapid attachment and increased spreading on substrates, morphological changes such as highly developed membrane systems, increased lysosomal hydrolases and increased capacity to kill intracellular parasites and tumour cells. Yet, there was little to distinguish between normal and BCG-activated macrophages in terms of handling *T. cruzi* 2 h after infection; the number of organisms ingested, and their uptake into phagocytic vesicles were very similar (Fig. 1a and b and Fig. 2). While some killing of parasites was detectable as early as 2 h, by 24 h after infection at a parasite-macrophage ratio of 1 most ingested parasites were undergoing degradation intracellularly both in normal and activated macrophage populations, although at a ratio of 10 the activated macrophages seemed to be more advanced in digesting the organisms. It has been reported that living toxoplasmas can survive within mouse macrophages because they block fusion of primary lysosomes with phagocytic vesicles⁹; consequently, the Thorotrast labelling technique was used to visualise lysosomes.

Fig. 1 Fate of *T. cruzi* in normal and activated macrophages. Peritoneal macrophages from normal mice and from animals primed and challenged with BCG (activated) were cultured at 2×10^6 – 3×10^6 cells per ml in 10% foetal bovine serum on coverslips in Leighton tubes. To label phagocytic vacuoles and lysosomes, 24 h before infection or collection, macrophages were treated with a thorium dioxide (Thorotrast) suspension at a final dilution of 1:175. After washing, cultures were infected with 7-d cultures, primarily epimastigote forms, of *T. cruzi* at 1:1, 10:1 and 100:1 parasite-macrophage ratios. After 2 h, coverslips were washed three times with agitation to remove free swimming forms, and replaced with fresh medium and observed at 2, 24, 48, 72, 96 and 192 h after infection. Details for preparation of coverslips for electron microscopy have been described elsewhere⁸. *a*, *c* and *e*, Normal macrophages; *b*, *d* and *f*, activated macrophages infected at a parasite-macrophage ratio of 10. *a* and *b*, Taken 2 h after infection (primary magnification $\times 2,200$). Arrows indicate dark Thorotrast-containing vesicles (lysosomes) which have fused with phagocytic vesicles containing trypanosomes in both normal and activated macrophages. *c* and *d*, Taken after 96 h of infection (primary magnification $\times 1,320$) and indicate persistence of trypanosomes in the normal macrophages but complete elimination from the activated macrophages. Note the relative abundance of Thorotrast-containing lysosomes and vacuoles and enlarged Golgi region. Arrows indicate trypanosomes residing in the cytoplasm. *e* and *f*, Higher magnifications ($\times 3,520$) of 96-h infected macrophages. *e*, Trypanosomes in the cytoplasm of normal macrophages. Note absence of trypanosomes in Thorotrast-marked vesicles. *f*, Trypanosomal remnants, including kinetoplast within Thorotrast-containing vesicle within an activated macrophage.



With *T. cruzi* virtually all parasites entered through phagocytic vacuoles which is consistent with the observation that cytochalasin B diminished the number of organisms found within macrophages⁹. Fusion of Thorotrast-labelled lysosomes and vacuoles with *T. cruzi*-containing phagocytic vesicles occurred equally well in normal macrophages and in BCG-activated macrophages (Fig. 1*a* and *b*). On the basis of observation of cells infected for 2–24 h, it would be impossible to predict which cultures were likely to be more effective in clearing the infection.

When infected cultures were examined 4–6 d after infection, however, a considerable difference was observed in *T. cruzi* growth in normal relative to activated macrophages (Fig. 1*c* and *d* and Fig. 2). Virtually all macrophages in the BCG-activated population infected at parasite-macrophage ratios of 10 had killed and degraded the intracellular parasites. The cytoplasm consisted of Thorotrast-labelled lysosomes, hyperactive Golgi and developed membranes, with occasional remnants of trypanosomes (Fig. 1*d* and *f*). In contrast, normal macrophage populations showed extensive intracellular growth of the *T. cruzi* (Fig. 1*c* and *e*). Significantly, ultrastructural examination revealed that the parasites were proliferating within the cytoplasm of the cells and were no longer found in phagocytic vesicles. Essentially no Thorotrast could be seen surrounding the parasites, and only the outer membrane of the parasite could be observed with the tilting EM stage separating them from the cell cytoplasm.

It thus seems that *T. cruzi* can survive in normal macrophages

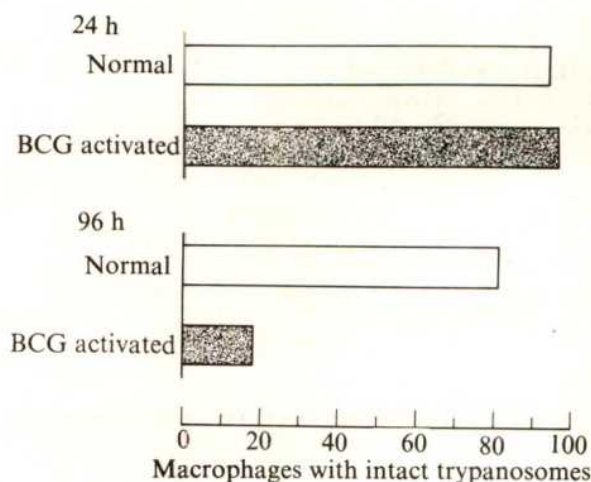


Fig. 2 Survival of intact *T. cruzi* in macrophages. Counts of intact trypanosomes were made on electron microscopic scanning of 100 consecutive macrophages from 24-h or 96-h cultures infected at a ratio of trypanosomes-macrophage of 10:1.

by escaping from phagocytic vacuoles into the cytoplasm where, presumably, macrophages have no specially developed mechanisms for killing. Even though most parasites are probably killed in normal macrophages, the small number which survive seem capable of escaping and proliferating intracytoplasmically. Nonspecific activation of macrophages, as by BCG, seems to enhance the efficacy of killing in these cultures.

The distinctness of the patterns of interaction between different protozoan parasites and host cells is emphasised by these studies. Although nonspecifically activated macrophages were more effective in killing *T. cruzi*, this has not been found

to be the case with *Toxoplasma gondii* unless specific antibodies¹⁰ or supernatants of activated lymphocytes^{11,12} were present. Mauel *et al.*¹³ observed that nonspecifically activated mouse macrophages were capable of enhanced killing of *Leishmania enriettii*, which is infectious for guinea pigs, but not mice, but not of *L. tropica*, which is infectious for mice.

Our results are consistent with the finding in bacterial systems⁷ that killing seems to be very rapid, whereas degradation may take longer. The only other instance of which we are aware in which protection against macrophage killing may be associated with growth of the organism free in the cytoplasm is that of human *Mycobacterium leprae*. In studies on the growth of the organism in lymph nodes draining foot pads of mice challenged with *M. leprae*, Evans and Levy^{14,15} observed that during the logarithmic growth phase of the organism, only one membrane seemed to separate the mycobacterium from the cytoplasm; in the plateau phase, presumably brought about by the development of cellular immunity in the host, it seemed that *M. leprae* was found in vacuoles containing an additional membrane, probably the lysosomal membrane. Together, these experiments emphasise (1) the importance of lysosomes in controlling intracellular parasitism; (2) the need to design chemotherapeutic agents able to act within lysosomes; (3) the possible efficacy of nonspecific, and ultimately of specific, immunisation in enhancing destruction of phagocytosed parasites.

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Suppression of cell-mediated tumour immunity by *Corynebacterium parvum*

THE use of immunoadjuvants in tumour therapy is based largely on the concept that an increased level of specific immunity against tumour-associated antigens may be achieved by nonspecific stimulation of the immune system (for review see ref. 1). Among these adjuvants, *Corynebacterium parvum* has received much attention since it represents one of the most powerful stimulants of the reticuloendothelial system in mice², and is effective in inhibiting tumour growth in several animal tumour systems (for example, see refs 3–6). The mechanisms by which *C. parvum* interferes with tumour growth have not been established and the tumour protective effect of *C. parvum* has not been entirely consistent in all systems studied. An immunosuppressive effect of *C. parvum* has been demonstrated too. In mice, the *in vitro* lymphoproliferative

Table 1 Effect of injection of *C. parvum* on the *in vitro* secondary cytotoxic response of MSV-immune spleen cells against specific targets

Experiment no.	Source of spleen cells*	Effector cell–target cell ratio		
		50:1	25:1	12.5:1
1	MSV 30	62.8	44.4	27.1
	MSV 30	58.6	38.1	16.9
	MSV 30, CP 7	16.5	9.2	7.3
	MSV 30, CP 7	13.1	9.3	4.5
2	MSV 30	80.6	70.3	50.8
	MSV 30	72.6	63.1	43.3
	MSV 30	75.5	62.6	44.6
	MSV 30, CP 7	24.7	18.4	10.8
	MSV 30, CP 7	28.7	17.2	11.2
	MSV 30, CP 7	26.6	15.0	10.9

*Mice were tested 30 d after injection of MSV and 7 d after intraperitoneal injection of 1.4 mg CP. A total of 20×10^6 spleen cells was cultured with 1×10^6 mitomycin C-treated RBL-5 ascites lymphoma cells for 5 d.

Surviving cells were counted by Trypan blue exclusion and the cytolytic capacity of the spleen cells against RBL-5 cells was tested in a 4-h ⁵¹Cr release assay of cytotoxicity (ref. 12) at various effector cell–target cell ratios. Results represent the mean percentage ⁵¹Cr release of quadruplicate samples of individual mice in two representative experiments. Spleen cells cultivated for 5 d in the absence of RBL-5 cells showed 5–10% cytotoxicity at the 50:1 ratio and less than 5% cytotoxicity at the two lower ratios.

responses to mitogens and to alloantigens were depressed after injection of *C. parvum*. It remains to be determined, however, whether a defect measured by these general tests of T lymphocytes may also represent an indication of depressed cell-mediated immunity against tumour cells.

A specific secondary *in vitro* cytotoxic response of immune spleen cells has been demonstrated in a murine sarcoma, virus (Moloney)–(MSV)–induced tumour system⁸. The secondary response was much stronger than the primary cytotoxic response in this tumour system and in its kinetics correlated better with the status of *in vivo* immunity as determined by transplantation protection studies. We now report that injection of CP markedly inhibited the ability of spleen cells to undergo an *in vitro* secondary cytotoxic response, suggesting that treatment with CP causes a depression of specific anti-tumour cellular immunity.

Primary MSV tumours were induced in C57BL/6N mice by intramuscular injection of virus (all details of this tumour system as used in our laboratory are given in refs 9–11). Tumours usually regressed after 21 d and mice were then immune to rechallenge with MSV or cross-reacting leukaemia cells for extended periods. A secondary cytotoxic response of immune lymphocytes could be elicited either *in vivo*¹² or *in vitro*⁸. For testing the *in vitro* secondary cytotoxic response, spleen cells from mice 30 d after virus injection (designated MSV 30 spleen cells) were cultivated in the presence of mitomycin C-treated ($100 \mu\text{g ml}^{-1}$, 30 min) RBL-5 ascites lymphoma cells which have been shown to possess the relevant antigens of the MSV system¹⁰. A total of 20×10^6 spleen cells and 1×10^6 RBL-5 cells was cultivated for 5 d in 5 ml of medium RPMI 1640 (supplemented with 5% foetal bovine serum and 5×10^{-5} M 2-mercaptoethanol) in 16-ounce tissue culture flasks. Subsequently, the sensitised spleen cells and appropriate controls were tested for their ability to lyse RBL-5 cells in a 4-h ⁵¹Cr release assay (details of this assay are in ref. 9).

As Tables 1–3 show, a vigorous cytotoxic response of MSV 30 spleen cells could be demonstrated after stimulating them *in vitro* with mitomycin C-treated RBL-5 cells. There was only little activity when MSV 30 spleen cells were cultivated for 5 d in the absence of RBL-5 cells (Tables 2 and 3). The response of normal spleen cells to RBL-5 cells was also very low in our culture conditions (data now shown).

To investigate the effect of *C. parvum* (formalin-killed CP vaccine (N6314) batch 1 PX 398, Burroughs Wellcome, Research Triangle Park, North Carolina) on the *in vitro* secondary

cytotoxic response of MSV 30 spleen cells, two different protocols were used. In the first, 1.4 mg *C. parvum* was injected intraperitoneally into MSV-immune mice 23 d after virus inoculation and spleen cells were tested 7 d later for their activity in the *in vitro* assay of the secondary cytotoxic response. Representative experiments are shown in Table 1. Treatment by *C. parvum* decreased the cytotoxic response of immune spleen cells by more than 70%. This was equally observed at effector cell-target cell ratios of 50:1, 25:1, and 12.5:1.

In the other protocol, 1.4 mg *C. parvum* was injected intraperitoneally into normal C57BL/6 mice, and after 8–10 d their spleen cells were mixed with MSV 30 spleen cells, to determine their effect on the *in vitro* secondary cytotoxic response. Various numbers of spleen cells from mice injected with *C. parvum* were mixed with a fixed number of MSV 30 spleen cells. As controls, spleen cells from normal untreated mice were added. As Table 2 shows, the addition of normal spleen cells at three different doses had little or no suppressive effect on the cytotoxic response of MSV 30 spleen cells. A significant depression was seen, however, when *C. parvum* spleen cells were added to MSV 30 spleen cells.

Table 2 Effect of spleen cells from *C. parvum*-injected mice on the *in vitro* secondary cytotoxic response of spleen cells from mice immune to MSV

Source and number of cells added to 20 × 10 ⁶ MSV 30 spleen cells*	Stimulating cells	
	None	RBL-5
None	8.3†	75.8
40 × 10 ⁶ normal spleen cells	6.6	63.7
20 × 10 ⁶ normal spleen cells	7.9	68.8
10 × 10 ⁶ normal spleen cells	8.1	73.2
40 × 10 ⁶ <i>C. parvum</i> spleen cells	6.1	18.3‡
20 × 10 ⁶ <i>C. parvum</i> spleen cells	7.7	30.6‡
10 × 10 ⁶ <i>C. parvum</i> spleen cells	7.8	50.7‡

*CP spleen cells were used 8 d after intraperitoneal injection of 1.4 mg into normal C57BL/6 mice. Normal spleen cells or *C. parvum* spleen cells were cocultivated with MSV 30 spleen cells for 5 d *in vitro* in the presence or absence of mitomycin C-treated RBL-5 cells and subsequently tested for their ability to lyse RBL-5 cells in a 4 h cytotoxicity assay at a 50:1 effector cell-target ratio.

†Mean % ⁵¹Cr release of quadruplicate determinations.

‡Significant (*P* < 0.05) suppression by *C. parvum* spleen cells when compared with the effect of the same number of normal spleen cells.

To investigate the nature of the suppressor cells in *C. parvum* spleens, *C. parvum* spleen cells were added to MSV 30 spleen cells after pretreatment with various techniques. These have been described in detail previously^{11,13}, and included treatment by anti-θ serum plus complement, by an iron/magnet technique, by rayon adherence columns, and by 2,500 rad X irradiation. Controls consisted of normal spleen cells treated by the same

techniques. The results of these experiments are summarised in Table 3. In four different experiments, a marked secondary cytotoxic response was seen in cultures of MSV 30 spleen cells which ranged between 64.3 and 78.4%. Addition of an equal number of normal spleen cells, either untreated or treated by any of the techniques mentioned above had little or no inhibitory effect. In contrast, *C. parvum* spleen cells consistently suppressed the cytotoxic response of MSV 30 spleen cells. Their effect was eliminated by pretreatment with the adherence columns or with the iron/magnet technique. The suppressive capacity of *C. parvum* spleen cells was not removed by pretreatment with anti-θ serum plus complement or by irradiation with 2,500 rad.

These results indicate that the cells in *C. parvum* spleens which inhibit the secondary cytotoxic response of immune spleen cells to tumour-associated antigens are radioresistant non-T cells. A role of B cells has not been definitively ruled out. The iron/magnet technique and the adherence columns as used in our laboratory, however, although effective in removing macrophages, do not selectively deplete B lymphocytes (this was shown both by stimulation with the B cell mitogen LPS and by determination of cells with detectable surface immunoglobulin). Therefore, our data suggest that the suppressor cells in *C. parvum* spleens are macrophages. This is in accord with the results of studies of the suppression of mitogen responses of normal spleen cells by *C. parvum* spleen cells⁶, in which the suppressor cells within *C. parvum* also appeared to be macrophages. In other studies, activated macrophages have been demonstrated after injection of *C. parvum* which inhibited the *in vitro* proliferation of tumour cells^{6,14}. Subsequently, we have presented evidence that in spleens of *C. parvum*-injected mice the same cells may suppress mitogen reactivity and inhibit tumour cell proliferation *in vitro* (H.K. *et al.*, unpublished). It is conceivable that activated macrophages have the same dual effect *in vivo* after injection of *C. parvum*. There may be a balance between immunosuppression and the inhibition of tumour cell growth, and it is possible that treatment schedules with the greatest activation could result predominantly in immunosuppression and consequent facilitation of tumour growth. Indeed, in one experimental tumour system, strong tumour protection occurred in conditions of minimal systemic activation, whereas there was little protection after systemic administration of *C. parvum*⁶. This lack of protection may be caused by similar suppressor cells as described here. Furthermore, it has been observed that tumour protection by injection of X-irradiated tumour cells has been diminished in mice pretreated with *C. parvum*⁵.

In conclusion, our data show the depression of anti-tumour cellular immunity by treatment with *C. parvum*. These data are not necessarily at variance with reports in which a tumour-protective effect of *C. parvum* has been shown. *C. parvum* may

Table 3 Effect of various treatments on the suppressive effect of *C. parvum* spleen cells on the *in vitro* secondary cytotoxic response of MSV-immune spleen cells

Experi- ment no.	Type of treatment*	Type of spleen cells cultured									
		MSV 30 spleen cells alone†		MSV 30 spleen cells plus‡§ untreated <i>C.</i> <i>parvum</i> spleen cells		MSV 30 spleen cells plus treated <i>C.</i> <i>parvum</i> spleen cells		MSV 30 spleen cells plus untreated nor- mal spleen cells		MSV 30 spleen cells plus treated normal spleen cells	
		+ RBL-5	- RBL-5	+ RBL-5	- RBL-5	+ RBL-5	- RBL-5	+ RBL-5	- RBL-5	+ RBL-5	- RBL-5
1	Anti-θ serum plus complement	64.3¶	6.7	27.3	6.3	25.1	7.4	56.7	8.3	54.8	6.7
2	Iron/magnet	71.3	4.9	32.8	6.6	66.1	3.9	64.6	7.2	66.7	7.3
3	Rayon adherence columns	78.4	9.2	38.6	6.9	78.3	7.2	73.5	6.1	74.6	5.6
4	2,500 rad X irradiation	70.6	5.4	31.8	5.7	30.6	6.1	72.3	7.1	70.4	8.2

*Details of these treatments are contained in refs 14 and 17.

†MSV-immune spleen cells were obtained 30 d after MSV injection.

‡*C. parvum* spleen cells were obtained 8 d after injection of *C. parvum* into normal C57BL/6 mice.

§20 × 10⁶ MSV 30 and 20 × 10⁶ CP spleen cells were cultured for 5 d in the presence of mitomycin C-treated RBL-5 cells and subsequently tested for their ability to lyse RBL-5 cells in a 4-h ⁵¹Cr release assay of cytotoxicity. At least three different effector cell-target ratios were tested in each experiment. Shown here are the data at a 50:1 ratio.

¶Mean % ⁵¹Cr release of quadruplicate samples.

turn out to be a useful therapeutic agent in the treatment of cancer. Our data suggest, however, that there may be quite a delicate balance between the beneficial anti-tumour effects of *C. parvum* and its immuno suppressive effects. Considerable experimentation seems to be necessary to determine the various factors contributing to this complex situation. Therefore, caution should be exercised in using *C. parvum* for immuno-therapy of human cancer.

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Role of amino acids in osmoregulation of non-halophilic bacteria

MOST microorganisms show optimal growth at low osmotic pressures, but even so are often able to grow, though more slowly, in the presence of concentrations of environmental solutes sufficiently high to reduce the water activity to values as low as 0.86 (Table 1). The ability of osmophilic yeasts to grow at low water activities depends on the accumulation of high concentrations of polyhydric alcohols inside the cell¹, and similarly, halophilic bacteria accumulate potassium ions². Amino acids, especially proline, have been shown to stimulate growth³ and respiration⁴ of some bacteria at low water activities, and to accumulate in response to increased environmental sodium chloride⁵ and, more particularly, to osmotic dehydration (my unpublished results). I now report that growth of non-halophilic bacteria at low water activities seems to depend on the ability of the cell to balance the environmental osmotic pressure by intracellular accumulation of amino acids, and on the types of amino acid which accumulate.

Non-halophilic bacteria differ greatly in their tolerance to sodium chloride in the growth medium (Table 1, first and second columns). There are two possible explanations: (1) the cells are permeable to the solute and differences in tolerance reflect the intrinsic solute resistance of intracellular enzymes; (2) the cells are impermeable to the solute and must therefore compensate internally for the increased environmental osmotic pressure. To test the first possibility, the salt sensitivities of the enzymes, aldolase, isocitrate dehydrogenase and phosphohexose isomerase, were assayed in whole cell homogenates of bacteria with widely different salt tolerances (Table 2). Although differences were evident between species, these were not sufficient to explain the differences in halotolerance and, further, concentrations of sodium chloride which would totally inhibit all these enzymes *in vitro* still permitted growth of the intact cells of the three species tested. Complete permeation of solute into the cell therefore does not occur and a concentration

difference is maintained across the cytoplasmic membrane. Such a concentration difference between the inside and the outside of the cell would cause osmotically induced dehydration unless accumulation of some other osmotically active molecule occurred within the cell. An osmotically induced accumulation of proline in *Bacillus subtilis* had already been demonstrated (my unpublished results), and therefore analyses were made of the free amino acids within the cells of other species of bacteria which had been grown in the presence of high concentrations of sodium chloride. A concentration was chosen for each species which caused a 50% reduction in growth rate.

In each of the species tested, salt induced a characteristic increase in the levels of particular pool amino acids (Table 1, third and fourth columns). To balance 1 M NaCl outside the cell requires an internal concentration of approximately 1 M ionic, or 1.8 M non-ionic solute. In some instances the increase in intracellular amino acid concentration appeared insufficient to balance the external osmotic pressure (Table 1, fourth column). The probable explanation is, first, that bacteria are normally hypertonic with respect to the environment. (In fact the more osmotolerant a microorganism is, the greater is its hypertonicity, and more specifically its internal K⁺ concentration, when growing at high water activity⁶.) Consequently, a part of the added solute pressure need not be balanced by additional intracellular solute. Second, because glutamate is charged at neutral pH values, it is accompanied by a cation (usually K⁺), which also contributes to the osmotic balance. Third, and particularly for *Staphylococcus aureus*, Na⁺ may be imperfectly excluded and therefore contribute to the balance also.

That the changes in amino acid level are osmotically induced can be judged by the fact that the same qualitative changes are induced by sucrose in place of salt in the environment, but the increase of intracellular amino acid per mol of sucrose is roughly 45-50% of the increase per mol of sodium chloride.

What can induce these changes? They occur in a rich medium, when the cells transport amino acids from the medium, or in a minimal medium when they synthesise them *de novo*. Those organisms which accumulate glutamic acid in response to osmotic stress are mainly Gram-negative species which, under normal conditions, have low total amino acid pools composed almost entirely of glutamate and also low K⁺ levels. At the other extreme, those organisms which accumulate proline are Gram positive and have a large free amino acid pool under non-stressed conditions (of which, again, a large proportion is glutamate) and also a high internal K⁺ level. In fact, such Gram-positive organisms growing in the absence of osmotic stress have pools similar to those of Gram-negative organisms grown in the presence of, say, sodium chloride. It thus seems that, during osmotic stress, the fundamental reaction is an increase in pool glutamate. Among Gram-negative organisms there is scope for this increase, but among the Gram-positive organisms glutamate may already be at a limiting concentration. Accumulation of glutamic acid requires the cell to accumulate an accompanying cation in order to retain electrical neutrality. In most cases this is K⁺. The disadvantages of K⁺ accumulation to most species are the same as those of allowing NaCl to enter the cell—the inhibition of enzymatic processes. Gram-positive organisms have therefore evolved mechanisms to convert glutamate through two closely related pathways of metabolism to form the other osmoregulatory solutes, proline or γ -aminobutyric acid (GABA). Significantly, neither of these is highly charged at neutral pH value, and so will not require the presence of a neutralising cation.

Accumulation of K⁺ may also be involved in osmoregulation. The first effect of an osmotic stress is to remove water from the cell and thereby increase the intracellular K⁺ concentration. The enzyme involved in glutamate synthesis

Table 1 Relationship of the major osmoregulatory amino acids to osmotolerance of microorganisms

Organism	Minimum water activity* for growth in NaCl	Equivalent [NaCl] % w/v	Amino acid(s) showing major increase in free pool during growth in NaCl†	Increase in amino acid pool level per mol of environmental NaCl (mmol)‡	
<i>Pseudomonas aeruginosa</i>	0.970	5.1	Glutamic acid	900	
<i>Vibrio parahaemolyticus</i>	0.950	8.5	Glutamic acid	900	
<i>Escherichia coli</i>	0.950	8.5	Glutamic acid	150	
			GABA		300
			Proline		630
<i>Salmonella oranienburg</i>	0.950	8.5	Glutamic acid	520	
			Proline		500
<i>Clostridium sporogenes</i>	0.945	9.3	Glutamic acid	280	
			GABA		500
			Proline		420
<i>Lactobacillus plantarum</i>	0.945	9.3	Glutamic acid	420	
			Proline		830
<i>Bacillus megaterium</i>	0.945	9.3	Proline		1,330
<i>Serratia marcescens</i>	0.943	9.5	Glutamic acid	700	
			Proline		400
<i>Klebsiella aerogenes</i>	0.940	10.0	Glutamic acid	750	
			Proline		450
<i>Streptococcus faecalis</i>	0.940	10.0	GABA		750
			Proline		750
<i>Micrococcus lysodeikticus</i>	0.930	11.4	Proline		1,550
<i>Sarcina lutea</i>	0.920	13.0	Proline		1,570
<i>Bacillus cereus</i>	0.920	13.0	Proline		1,670
<i>Bacillus subtilis</i>	0.900	15.7	Proline		1,650
<i>Staphylococcus aureus</i>	0.860	20.6	Proline		1,050

* Microorganisms were grown with aeration at 37 °C in potato extract–yeast extract–glucose broth (Difco) with the following exceptions: *Vibrio parahaemolyticus* was grown in heart infusion broth (Difco); *Lactobacillus plantarum* in MRS broth (Oxoid); *Clostridium sporogenes* in reinforced clostridial medium (Oxoid) without aeration. Sodium chloride at various concentrations, as a solid, was added to exponentially growing cultures of the organisms and growth rate was determined by following absorbance at 580 nm. Minimum water activity for growth was then determined by extrapolating a graph of growth rate against sodium chloride concentration to zero growth rate. The water activity of this NaCl-containing medium was determined on a Sina equihygroscope.

† Microorganisms were grown at 37 °C in media containing sufficient sodium chloride to reduce the growth rate by 50%. At a concentration of 2×10^8 cells per ml cultures were collected by centrifugation and washed twice in 0.1 M phosphate buffer, pH 7.0, containing sodium chloride at a concentration the same as that in the growth medium. The cells were finally resuspended at a density of approximately 10^{10} per ml in ice-cold 5% (w/v) trichloroacetic acid. Amino acid analyses were carried out using a Technicon automatic amino acid analyser. For each species controls were grown to the same cell concentration in the absence of sodium chloride, then treated as above.

‡ Intracellular concentrations of amino acids are based on phosphate-impermeable volumes for each species.

in most bacteria in cultures not limited by ammonia is glutamate dehydrogenase, which catalyses the oxidative deamination of glutamate and (or) the reductive amination of α -ketoglutarate. Table 3 shows that this enzyme is activated by 500 mM K^+ up to tenfold in the direction of glutamate, but not at all in the reverse reaction in the several species tested. There is thus a cyclic build up of glutamate resulting from the osmotically effected increase in K^+ concentration leading to glutamate synthesis, which then requires inflow of cations to balance the charge, which further activate the enzyme. In organisms that accumulate glutamate, a concomitant increase in intracellular K^+ concentration is observed, and this is of the same magnitude as the increase in glutamate concentration. For example, in *Klebsiella aerogenes* grown in the absence of osmotic stress the intracellular K^+ concentration is 125 mM. During

growth in the presence of 1 M NaCl this increases to 625 mM, an increase of 500 mM. The extra glutamate accumulated under these conditions is 750 mM. Similar changes can be observed in other organisms.

In some bacteria, notably some *Bacillus* spp., glutamate synthesis occurs partly (or completely in the case of *Bacillus megaterium*) by a separate route, through glutamine synthetase (GS) and glutamine: α -ketoglutarate amidotransferase (GOGAT). The GS–GOGAT system is not activated by ions (Table 4), nor does the total enzyme level increase substantially during growth at low water activity and therefore other mechanisms must be involved. Ions may, for example, influence the enzymes of proline synthesis or inhibit feedback inhibition which normally limits the free proline pool level: and in some microorganisms the rate of transport of amino acids into the cell can be con-

Table 2 Inhibition of various microbial functions by sodium chloride

Organism	Growth*	Sodium chloride concentration (% w/v) causing 50% inhibition of:		
		Phosphohexose isomerase†	Isocitrate dehydrogenase‡	Aldolase§
<i>Escherichia coli</i>	4.4	3.75	3.5	3.75
<i>Bacillus subtilis</i>	8.1	5	5.25	4.75
<i>Staphylococcus aureus</i>	10.9	6	7	7

* Determined as in Table 1.

All enzyme assays were performed on whole cell lysates of the organisms prepared by ultrasonication of suspensions of 10^{10} cells per ml 0.1 M phosphate buffer, pH 7.0.

† Assayed according to the method of Bodansky and Calitri¹¹.

‡ Assayed according to the method of Siebert *et al.*¹².

§ Assayed according to the method of Sibley and Lehninger¹³.

Table 3 Effect of K⁺ ions on glutamate dehydrogenase activity

Organism	Substrate*	Activity† of enzyme in		
		0	250	500 mM K ⁺
<i>Escherichia coli</i>	NADPH	320	1,910	3,060
	NADP	80	75	70
<i>Klebsiella aerogenes</i>	NADPH	480	1,500	2,400
	NADP	65	65	60
<i>Bacillus subtilis</i>	NADPH	650	1,830	3,170
	NADP	25	20	20
<i>Staphylococcus aureus</i>	NADPH	400	1,520	2,810
	NADP	30	30	30

*NADPH oxidation was used as a measure of the forward reaction, NADP reduction as a measure of the reverse reaction. In the latter case 5mM glutamate was added in place of α -ketoglutarate in the standard assay procedure.

†Assayed according to the method of Meers *et al.*¹⁴.

Activities expressed in nmol NADP reduced (or NADPH oxidised) per min per mg protein.

Whole cell lysates were prepared as in Table 2.

trolled by the internal potassium ion concentration⁸. In all cases where GS-GOGAT substantially contributes to glutamate synthesis, however, the change in amino acid pool during growth at low water activity is not principally in glutamic acid. The accumulation of proline does not require the accumulation of a neutralising cation. In those organisms which do accumulate proline, no change in K⁺ concentration can be observed during growth under osmotic stress. For example in *Bacillus subtilis* the intracellular K⁺ concentration is 250 mM irrespective of the external NaCl concentration. This does not, however, mean that any osmoregulatory mechanism cannot involve K⁺. After an osmotic shock, and before the subsequent accumulation of amino acid, water is removed from the cell by the osmotic pressure difference. Because the cell is largely impermeable to many intracellular solutes, such solutes will remain inside the cell after osmotic shock, and their effective concentrations will increase. K⁺, of course, is among these solutes. Once amino acid concentration has begun to increase within the cell, water is drawn back into the cell also, and the effective concentration of the other intracellular solutes will decrease again.

Table 4 Effect of K⁺ ions on GS-GOGAT activity

Organism	Activity* of enzymes in		
	0	250	500 mM K ⁺
<i>Escherichia coli</i>	<10	10	10
<i>Klebsiella aerogenes</i>	10	15	10
<i>Bacillus subtilis</i>	25	25	20
<i>Staphylococcus aureus</i>	30	30	20

*Assayed according to the method of Meers *et al.*¹⁴.

Activities expressed as nmol NADPH oxidised per min per mg protein.

Similar osmoregulatory mechanisms to those described above operate in aquatic invertebrates⁹. Further, proline accumulation is important in plants that have evolved to resist dehydration¹⁰. Until the evolution of homoeosmotic mechanisms in higher animals this primitive mechanism may have been widespread throughout the biological world from the simplest prokaryotes through to higher plants and invertebrate animals.

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Heterologous reattachment of regular arrays of glycoproteins on bacterial surfaces

REGULAR arrays of macromolecular subunits have been observed on the surfaces of a wide range of bacteria in electron micrographs of negatively stained or freeze-etched preparations^{1,2}. The regular patterns have mostly tetragonal or hexagonal symmetry and the centre-to-centre spacing between adjacent subunits varies from 6 to 15 nm. Chemical analyses of isolated subunits have shown that they are composed mainly of protein¹ or glycoprotein (unpublished) with molecular weights ranging from 65,000 to 150,000. Studies of a few bacteria have shown that the isolated subunits can assemble spontaneously to form regular arrays with the same dimensions as those seen in intact bacteria³. In appropriate conditions the subunits also reattach to the surfaces of cell walls from which they have been detached. Little is known about the biological function of these regular arrays of macromolecules on bacterial surfaces. That they cover the surface, leaving no gaps², suggests that they are important, for instance, in providing protection against adverse environmental conditions. Otherwise they would be expected to have been lost during evolution.

I have examined the ability of isolated subunits to reattach to cell surfaces to understand more fully the mechanisms involved in the development and maintenance of a two-dimensional array of macromolecules on a growing cell surface, and to obtain evidence for the suggestion that the assembly of these arrays is a dynamic process². Two strains of taxonomically closely related hyperthermophilic clostridia were used (grown at 65-70 °C), *Clostridium thermosaccharolyticum* and *C. thermohydrosulfuricum*⁴, which have tetragonal and hexagonal surface patterns, respectively (Fig. 1). Various treatments were used to remove the subunits from the surface of isolated cell walls and complete removal was only obtained with H-bond dis-

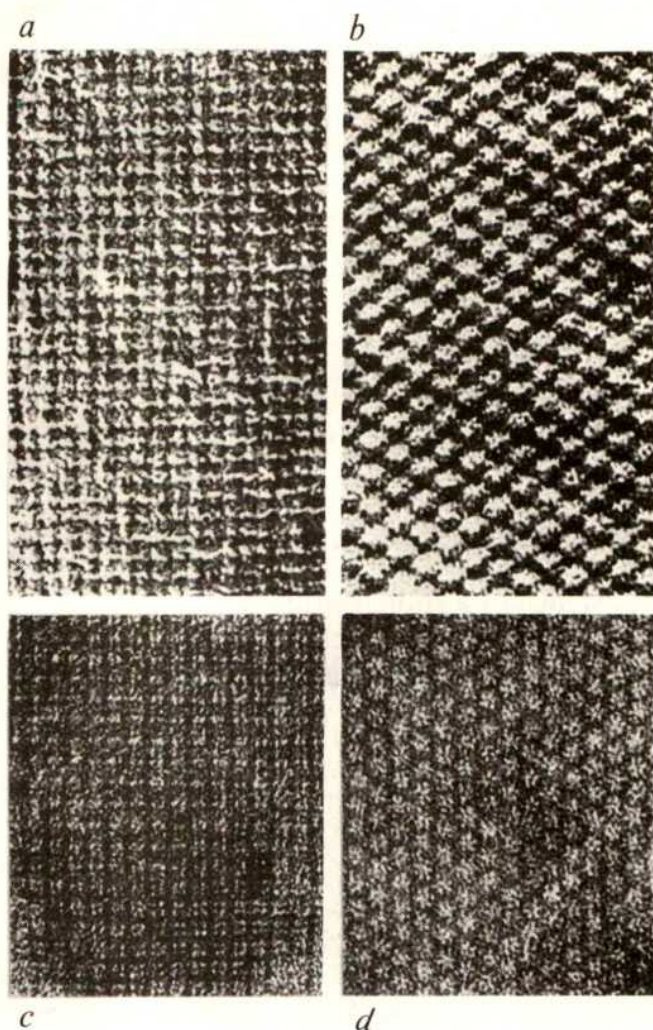


Fig. 1 Electron micrographs of freeze-etched preparations of the intact cells showing a portion of *a*, the regular tetragonal arrays of subunits on the surface of *C. thermosaccharolyticum* and *b*, the hexagonal arrays on *C. thermohydrosulfuricum*. ($\times 240,000$.) Electron micrographs of preparations of cell walls from *c*, *C. thermosaccharolyticum* and *d*, *C. thermohydrosulfuricum* negatively-stained with uranyl acetate. Cells were disrupted in an Aminco French pressure cell, and clean cell walls were isolated by treatment with Triton X-100 to remove the plasma membranes and washing in Tris-HCl (50 mM, pH 7.4) buffer. ($\times 240,000$.)

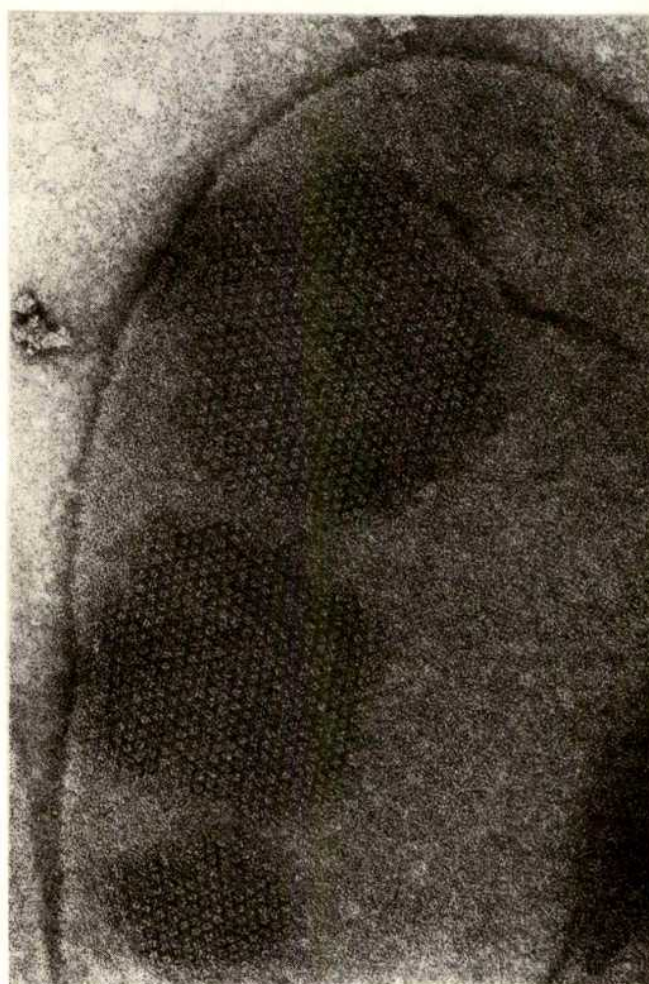
rupting agents, such as urea (8 M) and guanidine hydrochloride (GHCl, 5M). EDTA, Triton X-100, dithiothreitol and proteolytic enzymes, such as papain, trypsin, Pronase and thermolysin, had no visible effect on surface patterns. Analysis of treated cell walls and urea and GHCl extracts by sodium dodecyl sulphate (SDS)-polyacrylamide electrophoresis showed that the subunits of the surface layer form a major band which stains for both protein and polysaccharide (U.B.S., and K. J. I. Thorne, unpublished). The subunit protein of both microorganisms has a predominantly acidic amino acid composition and an acidic isoelectric point after isoelectric focusing on polyacrylamide gels. The molecular weights were 140,000, as determined by SDS-polyacrylamide electrophoresis.

Acidic conditions had an unexpected effect on the surface pattern. When cell walls were treated with buffer at a pH lower than 3, the regular arrays of subunits were no longer visible in negatively stained or freeze-etched preparations, and no periodicity was detectable in optical diffraction analyses of electron micrographs. No protein was lost, however, and the regular patterns reappeared very clearly when the pH was adjusted to neutral. Consequently it was con-

cluded that acidic conditions cause an uncoiling of the surface glycoprotein but do not detach it from the cell surface. When urea or GHCl extracts were dialysed against buffer at neutral pH (Tris-HCl, 50 mM, pH 7.4) the isolated subunits of both organisms could assemble into regular arrays *in vitro* in the absence of any supporting layer. This process was reversible, and the self-assemblies could be disintegrated and reformed by lowering and raising the pH, down to 2-3 and up again to 7. High salt concentrations (3 M KCl) had no effect on the self-assemblies. These chemical studies show that covalent bonds are not involved in the linkage between the subunits in the regular array, or between the subunits and the surface layer of the cell wall.

Isolated subunits reattach to cell walls in various conditions, such as dialysis of a mixture of urea or GHCl extract with 'naked' cell walls against distilled water or buffer. The reattached subunits form small areas of regular pattern, or crystallites, at various orientations (Fig. 2), and not the large regular arrays observed on intact cells. An unexpected finding was that subunits from one organism can attach to cell walls of the other organism and form patterns identical to those on the cells from which they originated. In addition, when cell walls from one organism are incubated with a mixture of both types of subunit, regular arrays, some with hexagonal and some with tetragonal symmetry, were observed on individual cell walls (Fig. 3). The two patterns were observed with equal frequency and the cell walls did not seem to favour the attachment of one type of subunit over the other. These observa-

Fig. 2 Negatively stained preparation of a fragment of cell wall from *C. thermohydrosulfuricum* which was incubated with subunits isolated from the same organism. The reattached subunits form small areas of hexagonal pattern. ($\times 120,000$.)



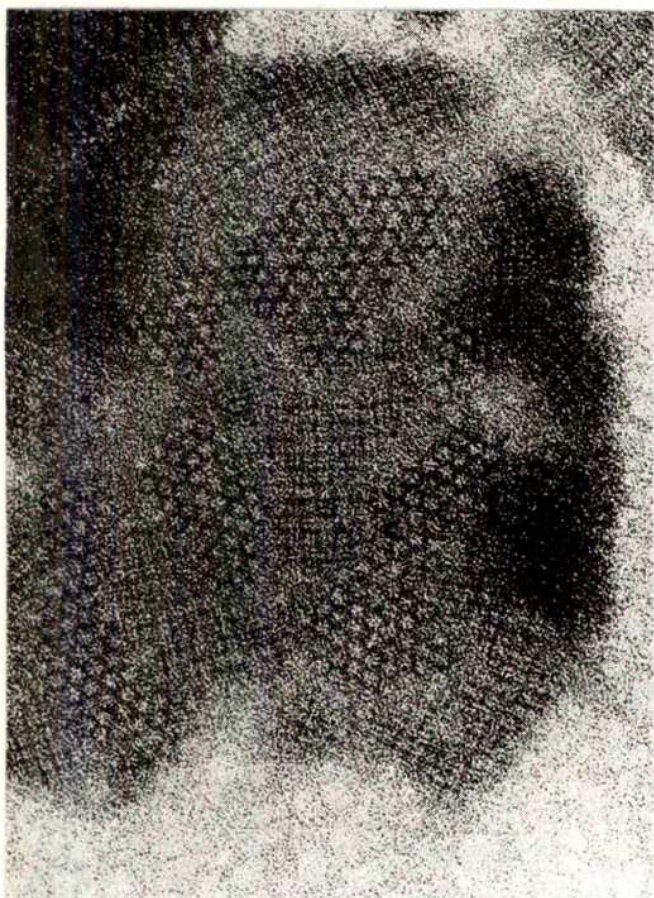


Fig. 3 Negatively stained preparation of a fragment of cell wall from *C. thermosaccharolyticum* which was incubated with a mixture of both types of isolated subunit. Small arrays of both hexagonal and tetragonal patterns are visible. ($\times 180,000$.)

tions clearly demonstrate that the information for the formation of the regular patterns resides in the subunits themselves, and is not affected by the supporting cell wall layer. In addition, the crystallites are orientated at random on the cell walls, suggesting that their orientation is not determined by any pattern in the binding sites in the cell wall.

An analysis of freeze-etched preparations of regular arrays of subunits on logarithmically growing cells of both clostridia, and other studies on thin sections (unpublished), have shown that large numbers of newly formed subunits appear at sites of division. These subunits first form a mosaic of small crystallites and subsequently rearrange themselves into large areas of uniform pattern during cell separation. From these observations it was concluded that the subunits undergo a dynamic process of assembly during cell growth². Such a process also requires a non-orientating supporting layer which allows free rotation and movement of subunits into the regular pattern. Thus it seems that the pattern is only determined by the directional bonds between the subunits and presumably represents the arrangement in which there is least strain and minimum energy in these bonds. The ability of the subunits to self-assemble into regular arrays and to adhere readily to the cell surface provides a simple mechanism for keeping a growing surface completely covered with a monolayer of macromolecules, providing that an excess of subunits is available either inside or outside the cell. The alternative mechanism of growth of a surface layer composed of regular arrays of macromolecules by the migration of dislocations⁹ is much more complex since it requires both the precise incorporation of subunits at particular points on the surface and that the rate

of glycoprotein synthesis be synchronised with the rate of synthesis of the underlying cell wall.

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Existence of a follicle-stimulating hormone inhibiting factor 'inhibin' in bull seminal plasma

THE existence of a substance of testicular origin capable of inhibiting the secretion of pituitary follicle-stimulating hormone (FSH) was postulated by McCullagh¹, who gave it the name 'inhibin', and direct, though not conclusive, evidence for its presence has since been provided²⁻⁶. After extraction and gel chromatography of bull seminal plasma, Franchimont *et al.*^{7,8} obtained a proteinaceous fraction (AcII) capable of decreasing basal FSH levels as well as diminishing the FSH response to exogenous luteinising hormone releasing hormone (LHRH) in normal and castrated adult male rats. We here summarise work based primarily on the specific biological effects of antiserum to AcII in rats, suggesting the existence of inhibin in bull seminal plasma.

Gel chromatography of bull semen (see Table 1) yielded a major unretarded peak (AcI: 200 ± 10 mg) followed by another distinct peak (AcII: 45 ± 5 mg) with a trailing edge. When 200- μ g aliquots of AcII were analysed by polyacrylamide gel electrophoresis⁹ (pH 8.6; gel concentration 10%), it was resolved into at least three components, as revealed by staining with Amido black.

AcI caused no significant decrease in serum FSH or LH in castrated adult male rats at a dose of 500 μ g per animal^{7,8}, whereas 200 μ g of AcII, intraperitoneally or intravenously, caused a consistent and significant decrease in serum FSH without affecting LH (Table 1). With normal intact males, a dose-dependent decrease in FSH was obtained after intraperitoneal injection (experiment 1, Table 1), although pituitary weight and gonadotrophin content were not affected.

The active fraction was examined for the possible presence of specific androgen binding protein (ABP) with high affinity for testosterone and dihydrotestosterone (DHT)¹⁰. Samples (100 and 200 μ g) were equilibrated at 0 °C for 2 h with tritiated testosterone, DHT and 17- β -oestradiol¹⁰. Proteins were fractionated by polyacrylamide gel electrophoresis and the radioactivity measured in 2.5-mm thick slices of the gel¹¹. No steroid-binding capacity for testosterone, DHT or 17- β -oestradiol could be detected in any of the three components of AcII. Thus, inhibin is neither identical nor similar to ABP elaborated by Sertoli cells under the influence of FSH¹⁰. The possibility that the biological effect of the active material was caused by contamination with steroids or their conjugates, was excluded by radioimmunoassay for testosterone, progesterone and 17- β -oestradiol after appropriate organic solvent extraction¹². None of these steroids

Table 1 Effect of seminal plasma fraction AcII on serum FSH and LH levels in adult male rats

Experiment	Treatment	Injection route	No. of rats	Serum FSH (ng ml ⁻¹ ± s.e.m.) NIAMD-FSH-RP ₁	Serum LH (ng ml ⁻¹ ± s.e.m.) NIAMD-LH-RP ₁
Normal rats	NaCl 0.9%	i.p.	10	581.0 ± 45.6	39.8 ± 7.1
	200 µg AcII	i.p.	10	423.0 ± 39.9*	39.1 ± 4.8
	500 µg AcII	i.p.	10	342.7 ± 44.6†	29.1 ± 4.6
	NaCl 0.9%	i.p.	5	638.0 ± 46	26.4 ± 5.3
	200 µg AcII	i.p.	5	458.0 ± 49.1*	20.0 ± 5.4
	NaCl 0.9%	i.v.	10	425.7 ± 30.8	48.9 ± 7.1
	160 µg AcII	i.v.	10	315.0 ± 32.2*	62.6 ± 8
	200 µg AcII	i.v.	10	1,261.0 ± 96	293.0 ± 25
Castrated rats	NaCl 0.9%	i.v.	10	855.0 ± 47.2†	320.0 ± 41.1
	200 µg AcII	i.v.	10	805.0 ± 31.4†	289.0 ± 45
	500 µg AcII	i.v.	8	1,080.0 ± 74.1	248.3 ± 61.7
	NaCl 0.9%	i.p.	10	795.0 ± 50.6*	324.2 ± 70.9
	200 µg AcII	i.p.	10	824.0 ± 83.9	421.0 ± 90.2
	NaCl 0.9%	i.p.	5	588.0 ± 56.2*	260.0 ± 22.7
	200 µg AcII	i.p.	5		
	200 µg AcII	i.p.	5		

Male albino Wistar rats (180–200 g) were given the total dose as four intraperitoneal (i.p.) injections at 12-h intervals. Rats were killed 4 h after the last injection, and blood and pituitaries collected. Alternatively, the test material or saline was administered as a single dose intravenously (i.v.) by way of the tail vein. Animals were autopsied 1 h later. Castrated animals were used 15 d after surgery. Serum samples were assayed for FSH and LH by radioimmunoassay using NIAMD systems¹⁸. Freshly collected bull semen was centrifuged at 4 °C to remove spermatozoa, and protein from the supernatant precipitated by addition of alcohol to a concentration of 86% (v/v). The precipitate was recovered by centrifugation in the cold, dissolved in distilled water and lyophilised. Batches of 300 mg were subjected to gel chromatography on Sephadex G100 in columns with a packed dimension of 2.5 × 30 cm, using 0.05 M acetate buffer pH 4.0 for equilibration as well as elution.

**P* < 0.05; †*P* < 0.025.

was detected in as much as 1,200 µg AcII. The possibility that the observed action of AcII may be a result of the presence of gonadotrophins, their fragments or metabolites, was also excluded by the fact that AcII does not cross react with either FSH or LH from man, sheep, rabbit or rat in the corresponding radioimmunoassay^{13–16}. An antiserum raised against AcII (see below) failed to bind labelled gonadotrophins of different species.

What seems to be significant is the demonstration that antibodies to inhibin can be raised in rabbits by active immunisation (2 mg AcII per animal in 1 ml saline mixed with an equal volume of Freund's complete adjuvant injected intradermally at several sites, followed by four booster intramuscular injections at 10–12-d intervals of 1 mg per animal in Freund's complete adjuvant; 15 d after the last injection the animals were bled by way of the carotid artery and sera recovered). The antiserum thus obtained is able to induce a marked increase in plasma FSH when injected into male or female rats.

The antiserum provoked a significant increase in the serum FSH levels in intact adult male rats when injected subcutaneously once a day for 4 d at doses of 0.5, 0.8 and 1.0 ml per animal, whereas serum LH, blood testosterone and testis and accessory organ weights were not significantly increased (Table 2). Nor did the antiserum lead to any histological changes in the seminiferous tubules.

In adult female rats, a dose of 1 ml antiserum per animal

Table 2 Effect of antiserum to seminal plasma fraction AcII on adult male rats

Treatment	No. of rats	Serum FSH (ng ml ⁻¹ ± s.e.m.) NIAMD-FSH-RP ₁	Serum LH (ng ml ⁻¹ ± s.e.m.) NIAMD-LH-RP ₁
Normal rabbit serum	25	320.7 ± 85.05	104 ± 69.5
0.2 ml Anti-AcII serum	5	306.0 ± 51.7	156 ± 76.3
0.4 ml	5	332.0 ± 66.4	121 ± 47.3
0.5 ml	5	958.8 ± 144.2*	209 ± 105.5
0.8 ml	5	1,570.0 ± 100.9*	187 ± 46.6
1 ml	5	1,420.0 ± 134*	290 ± 182.8

Antiserum to AcII was injected subcutaneously to adult male Wistar rats (180–200 g) once a day for 4 d, in graded doses (0.2–1.0 ml total dose) all made up to the same volume with saline; 12h after the last injection animals were killed for collection of blood, pituitary and reproductive organs. Pituitary and serum FSH and LH and serum testosterone were measured by radioimmunoassay.

**P* < 0.001.

Table 3 Effect of antiserum to seminal plasma fraction AcII on adult and immature female rats

Treatment	No. of rats	Serum FSH- (ng ml ⁻¹ ± s.e.m.) NIAMD-FSH-RP ₁	Serum LH (ng ml ⁻¹ ± s.e.m.) NIAMD-LH-RP ₁
Adult rats			
Normal rabbit serum	5	203 ± 44.8	86.3 ± 34
Anti-AcII serum 1 ml per rat	5	3,120 ± 261.9†	715 ± 49.9*
Immature rats			
Normal rabbit serum, 0.5 ml per rat	6	330 ± 44.1	135.8 ± 33.1
Anti-AcII serum, 0.5 ml per rat	6	8,900 ± 517.9†	1,808 ± 291*

Adult (180–200 g) and immature (21–24-d-old) female Wistar rats were treated with a total dose of 1.0 or 0.5 ml antiserum to AcII according to the schedule described in Table 2. Pituitary and serum gonadotrophin and serum 17-β-oestradiol were measured by radioimmunoassay.

**P* < 0.005; †*P* < 0.001.

significantly elevated both FSH and LH levels without affecting serum 17-β-oestradiol, ovarian or uterine weights (Table 3). Administration of antiserum to immature female rats resulted in an even more pronounced elevation of both FSH and LH (Table 3). The antiserum had no effect on the pituitary gonadotrophin content in male or female rats. The FSH response to 1 ml antiserum was more striking in the adult female (1,536% control) than in the adult male (443% control), which could be a reflection of the different sensitivities of the male and female hypothalamo-hypophysial axis to inhibin. The apparent FSH specificity of responses to antiserum in male animals may be dose dependent: higher doses may also stimulate LH release in much the same way as small doses of LHRH selectively increase LH, whereas increasing doses stimulate the release of both FSH and LH in adult human subjects¹⁷.

Our observations, particularly those relating to the effect of antiserum to AcII, clearly demonstrate the existence in bull seminal plasma of a proteinaceous substance capable of diminishing circulating levels of gonadotrophins, especially FSH, in male and female rats. This substance, which corresponds with the definition of inhibin, may be important not only in the control of spermatogenesis but also of the oestrous cycle. In the light of the effects of the antiserum to AcII in immature females, the possibility

exists that this substance may also be important for the transition of females from sexual immaturity to adulthood.

Observations on the differential effects of normal and azoospermic human seminal plasma on FSH levels in adult, castrated rats² clearly implicate the germinal epithelium as the source of inhibin. Setchell and Sirinathsinghji³ pointed out that the logical route out of the testis for such a substance would be by way of the rete testis fluid (RTF) and the epididymis. Although a substantial part may be reabsorbed, together with RTF in the head of the epididymis, it would not be unusual to find this substance in the ejaculate. Viewed in this light, it is tempting to speculate that the source of inhibin in bull seminal plasma is indeed the germinal epithelium of the testis; however, we do not as yet have any evidence bearing directly on this possibility.

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Brain lesions and precocious puberty in rats

IN the immature female rat, the ability to secrete the gonadotrophins luteinising hormone (LH) and follicle-stimulating hormone (FSH) in response to a single injection of oestradiol or of progesterone first appears about day 28 of life^{1,2}. Younger animals respond to these hormonal stimuli with an increased release of gonadotrophins only if they have been primed with an injection of oestrogen 3 or more days earlier, and it is assumed that the priming steroid promotes the functional maturation of a positive (stimulatory) feedback system³ by an unknown mechanism. Alternatively, electrolytic lesions in the area of the basal hypothalamus of 23-d-old rats induce precocious sexual maturation leading to pubertal ovulation within 4 or 5 d (ref. 4). The lesion site is rich in luteinising hormone releasing hormone⁵, and it seems likely that the discharge of this polypeptide triggers the rapid and sustained increase in the production of ovarian steroids, particularly of oestrogen, observed during this period⁴. We report here that the increase of endogenous steroid in response to a brain stimulus can prime the positive feedback system in a manner closely related to the effect of exogenous oestrogen. This suggests that brain lesions advance the process of sexual maturation through the precocious establishment of a positive feedback loop.

Female Sprague-Dawley rats were kept on a 12/12 h light/dark cycle (lights on at 0700). On day 23 of life, at about noon, animals weighing > 56 g either received an injection of 10 µg oestradiol benzoate (OB) in oil (0.2 ml, subcutaneously) or

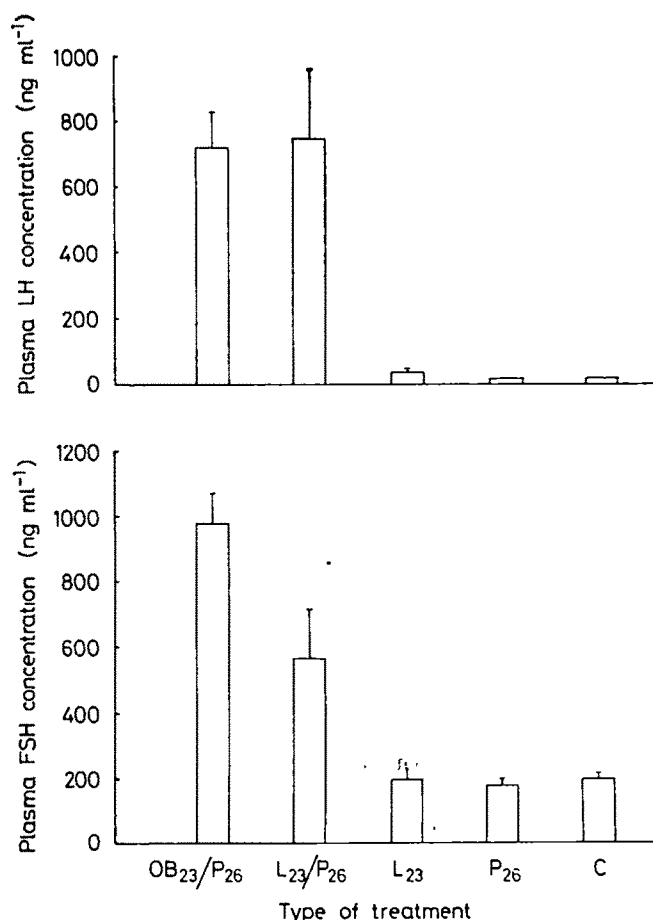


Fig. 1 Plasma LH and FSH concentrations 5 h after an injection of progesterone (P) in rats primed with oestradiol benzoate (OB) or with a brain lesion (L) and in controls. Samples were run in duplicate. Results are expressed as mean \pm s.e. in terms of NIAMDD-rat LH-RP-1 and FSH-RP-1 reference preparations. Control values of LH falling below the detection limit of the assay were considered to be 15 ng ml⁻¹. C, untreated controls. Indices refer to age (in d) at which treatment was applied.

were subjected to a small unilateral electrolytic brain lesion placed stereotactically near the origin of the pituitary stalk⁴. Seventy-two hours later the rats received 1 mg of progesterone in oil (0.2 ml, subcutaneously); they were decapitated 5 h later (that is at 1700 on day 26). Plasma was assayed for LH and FSH concentrations using NIH radioimmunoassay kits. Controls were either untreated littermates of similar body weight, were subjected to brain lesions alone or were injected with progesterone only on day 26.

Figure 1 illustrates that both types of sequential treatment elevated mean plasma LH concentrations more than 20-fold compared with all types of controls ($P < 0.005$; Student's *t* test). FSH concentrations were significantly ($P < 0.05$) higher

Table 1 Degree of uterine hypertrophy induced by oestrogen/progesterone treatment or by sequence brain lesion/progesterone compared with controls

Group	n	Treatment	Uterine weights (mg; mean \pm s.e.)	Significance (two-tailed <i>t</i> test)
1	8	OB ₂₃ /P ₂₆	150.9 \pm 5.9	1 against 2, $P > 0.2$
2	8	L ₂₃ /P ₂₆	140.0 \pm 12.6	
3	8	L ₂₃ only	104.5 \pm 14.6	3 against 4, $P > 0.2$
4	8	OB ₂₃ only	121.3 \pm 3.6	
5	8	P ₂₆ only	32.1 \pm 1.9	
6	8	None	36.7 \pm 5.5	

n, Number of animals per group. Rats were killed at 1700 on day 26, and uteri were dissected on filter paper. Indices as in Fig. 1.

after the combined hormonal treatment than after the sequence brain lesion/progesterone, but plasma values found after the latter approach nevertheless significantly ($P < 0.05$) exceeded control values in all groups. LH and FSH concentrations in controls were low, indicating that neither the brain lesion as such nor the injection of progesterone into unprimed animals can markedly increase plasma concentrations at this time.

The effect of the two treatments on uterine growth was compared (Table 1). The brain lesion (group 2) was found to substitute fully for the priming injection of OB (group 1) in this scheme; likewise, the degree of uterine hypertrophy induced by the lesion alone (group 3) was not significantly less than that seen after an injection of OB alone (group 4). In contrast, no uterine growth was induced when progesterone only was given on day 26 (group 5). From this cumulative bioassay, it is concluded that the brain lesion is equivalent to approximately 10 µg of injected OB.

The mode of action of brain lesions in the induction of precocious puberty is obscure⁶. It has been suggested that lesions eliminate the neural substrate which mediates the inhibitory action of gonadal steroids on the reproductive axis⁷. However, small unilateral lesions which spare most demonstrated oestrogen⁸ and progesterone⁹ uptake sites are efficient inducers of puberty⁴. Precocious sexual maturation can also be triggered, in rats, by repeated administration of oestrogen¹⁰ or by a single injection of pregnant mare serum gonadotrophin, and, in mice, by male-produced pheromones. The latter two mechanisms are dependent on the secretion of endogenous oestrogen^{11,12}. We submit that oestrogen also reinforces the stimulatory effect of the hypothalamic lesion.

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Rapid and dissociated changes in sensitivities of different dopamine receptors in mouse brain

DOPAMINERGIC systems in various parts of the mammalian brain, particularly in striatal, mesolimbic, mesocortical and hypothalamic structures, are involved in the control of motor activity, autonomic processes and emotional behaviour. An altered dopaminergic transmission in striatum seems to be responsible for Parkinsonism, whereas abnormalities in other, still poorly defined, dopaminergic synapses could participate in the aetiology of schizophrenia¹⁻³. Thus, there is considerable theoretical as well as therapeutical

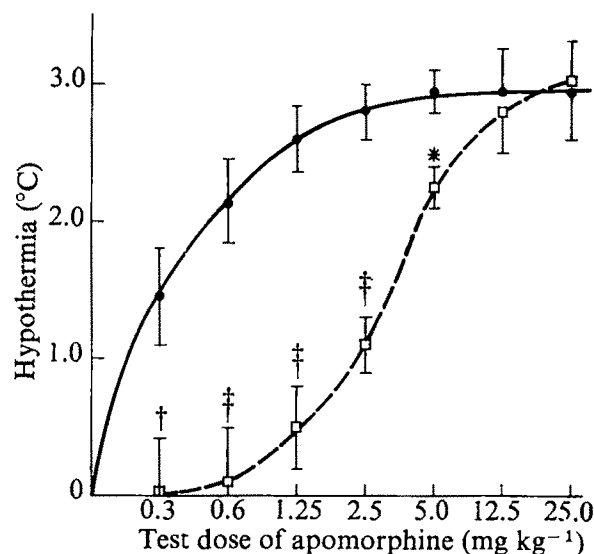
interest in defining specific characteristics of different dopaminergic systems participating in the control of these various functions.

Hypersensitivity to the action of specific dopamine agonists like apomorphine has been observed after either denervation⁴ or long term treatment with agents interrupting dopaminergic transmission⁵⁻⁷, whereas desensitisation occurs after chronic stimulation⁸. The study of these states of modified receptor sensitivity may contribute to the understanding of the various factors controlling dopaminergic transmission in normal and pathological conditions and may also be helpful in defining several classes of dopaminergic synapses.

We observed that the sensitivity of dopamine receptors involved in thermoregulation and in a particular motor behaviour can be rapidly altered after a single administration of moderate dosage of an agonist (apomorphine) or an antagonist (haloperidol), respectively; however, whereas dopamine receptors controlling thermoregulation are easily desensitised and do not develop supersensitivity, the reverse is true for those implicated in the motor behaviour.

Male Swiss albino mice (22-26 g, Charles River, Saint Aubin-lès-Elbeuf, France) were housed in a well ventilated room, at 24 °C and under artificial illumination (light on between 0800 and 2000). Three hours before the experiments the animals were housed without food in small individual cages at 22 ± 1 °C. Body temperature was measured with a thermoelectric probe (Ellab RM 6, Copenhagen) inserted 4 cm into the rectum, immediately before and at various intervals after subcutaneous injection of apomorphine. Apomorphine induced an hypothermia which was related to dose both in intensity and in duration. The maximal hypothermia in controls (saline-pretreated mice) was observed at about 5 mg kg⁻¹ of apomorphine (Fig. 1) and lasted for about 90 min. In mice pretreated with this dose and tested after 8 h, there was a significant shift to the right of the dose-response curve: although the maximal hypothermia (-3 °C) was not modified, the ED₅₀ for apomorphine was 2.80 ± 0.59 mg kg⁻¹ as compared with 0.35 ± 0.06 in controls (calculated by the method of Miller and Tainter⁹). On the other hand, the hypothermia induced by agents, such as oxotremorine (0.05 mg kg⁻¹), clonidine

Fig. 1 Modification in the apomorphine-induced hypothermia after a single administration of the drug. Dose-response curves were established 8 h after the subcutaneous injection of either apomorphine (5 mg kg⁻¹) or saline. The effects of test doses of apomorphine were recorded 30 min after subcutaneous injection. Means ± s.e.m. of 10-15 experiments for each dose. Means were compared by Student's *t* test. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.



(0.25 mg kg⁻¹) or promethazine (10 mg kg⁻¹), acting on other systems, was not modified in the apomorphine-pretreated animals.

In mice treated with an antagonist of dopamine receptors, the antipsychotic drug haloperidol (0.1 mg kg⁻¹, intraperitoneally), the hypothermic action of apomorphine (0.45 mg kg⁻¹) was blocked for at least 10 h. After 72 h, the dose-response curve to apomorphine of mice pretreated with haloperidol (either 0.1 or 4 mg kg⁻¹) was not significantly different from that of controls. Similarly, the responsiveness to a single dose of apomorphine (0.30 mg kg⁻¹) was not altered 4, 5, 7 and 10 d after haloperidol (4 mg kg⁻¹).

In addition, a peculiar motor behaviour, that is climbing or verticalisation induced by apomorphine (refs 7, 10, 11 and P.P., J.C. and J.C.S., unpublished) was evaluated. For that purpose, after drug administration, the mice were immediately put into individual cylindrical cages, 12 cm diameter, 14 cm high, with walls of vertical metal bars, 2 mm diameter, 1 cm apart, surmounted by a smooth surface. After a 5-min period of exploratory behaviour, whereas the controls remained on the floor of the cage, the apomorphine-treated mice tended to adopt a vertical position holding the bars for at least 30 min, either with their forefeet only (small doses of apomorphine) or with all four paws (larger doses). This behaviour was scored as follows: four paws on the floor (0), forefeet holding the wall (1), four paws holding the wall (2). Two observations were performed on each animal, 10 and 20 min after injection of apomorphine and the two scores averaged. In controls, the maximal score was obtained for about 1.0 mg kg⁻¹ of apomorphine with an ED₅₀ of 0.50 ± 0.04 mg kg⁻¹ (Fig. 2). In mice pretreated with haloperidol (4 mg kg⁻¹, intraperitoneally) 72 h earlier, the dose-response curve to apomorphine was shifted significantly to the left (ED₅₀ = 0.27 ± 0.02 mg kg⁻¹).

Although pretreatment with apomorphine resulted in a diminished sensitivity to its hypothermic effect, the same was not true for the climbing behaviour. In fact, 8 h after administration of apomorphine (5 mg kg⁻¹) the ED₅₀ was 0.11 ± 0.02 mg kg⁻¹ as compared with 0.45 ± 0.04 for animals pretreated with saline in the same experiment. This

Fig. 2 Modification in the climbing behaviour induced by apomorphine after a single administration of haloperidol. Dose-response curves (10–30 experiments for each dose) were established 72 h after the intraperitoneal injection of either haloperidol (4 mg kg⁻¹) or saline. Means were compared by the χ^2 test ($P < 0.001$). □, Haloperidol-pretreated mice; ●, saline-pretreated controls.

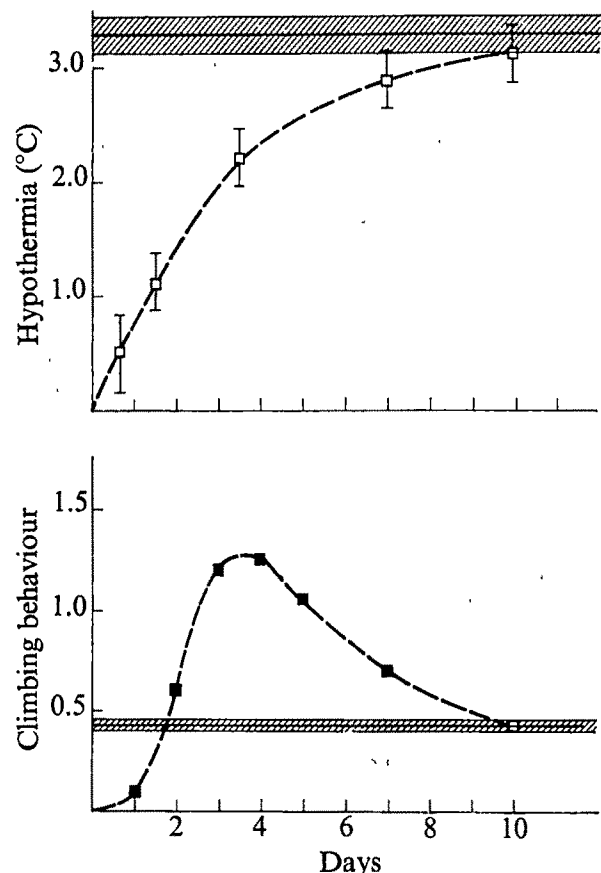
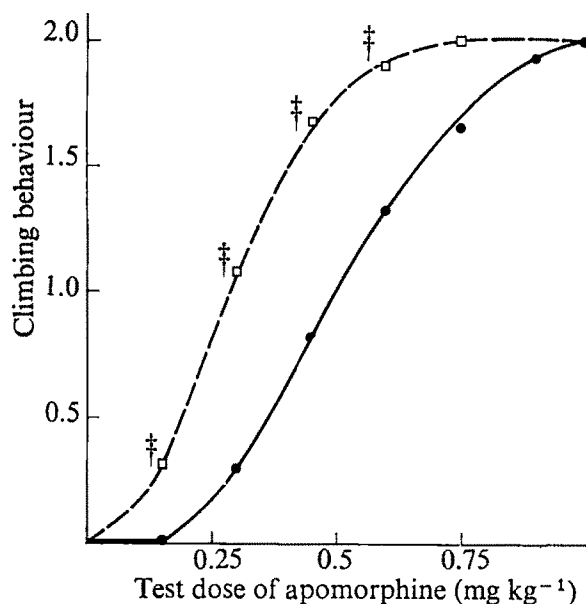


Fig. 3 Modification in the responses of mice to apomorphine at different times after a single pretreatment with either apomorphine (□, 5 mg kg⁻¹ subcutaneously) or haloperidol (■, 4 mg kg⁻¹ intraperitoneally). The intensity of the hypothermia in apomorphine-pretreated mice was determined 30 min after the subcutaneous injection of a test dose of 0.45 mg kg⁻¹ apomorphine (means of 15–30 experiments for each time interval). The climbing behaviour was scored after the subcutaneous injection of a test dose of apomorphine 0.30 mg kg⁻¹ (means of 10 experiments for each time interval). In both cases, the shaded area represents the mean ± s.e.m. of responses in saline-pretreated controls.

apparent facilitation of the climbing behaviour (which was also observed after a chronic treatment with apomorphine) could be due to the lack of hypothermia in these pretreated animals. This is suggested by a similar facilitation of the climbing behaviour, evaluated in naive animals, when they were kept at an environmental temperature of 30 °C instead of 22 °C, which compensates for the apomorphine-induced hypothermia.

Thus, it seems that a single administration of a drug acting at dopaminergic receptors is sufficient to modify the activity of apomorphine. It was therefore of interest to determine the onset and duration of the subsensitivity induced by apomorphine pretreatment and the hypersensitivity elicited by haloperidol (Fig. 3).

As early as 2 h after a dose of apomorphine (5 mg kg⁻¹, subcutaneously), when the normal temperature is reached again, a test dose of apomorphine (0.45 mg kg⁻¹) which, in controls decreased the temperature by 3 °C, had no effect. This refractoriness decayed progressively: it was still significant 4 d after treatment and completely disappeared only by 10 d. With regard to the climbing behaviour, the onset of the haloperidol-induced hypersensitivity could not be so precisely determined since the blockade by the antipsychotic lasted at least 24 h but there seemed to be no time lag between blockade and appearance of hypersensitivity (Fig. 3). Hypersensitivity was maximal between 72 and 96 h and also ceased by 10 d.

Modifications in the sensitivity of dopamine receptors

probably account for the modified responsiveness to apomorphine. The alkaloid is recognised as a specific agonist of dopamine receptors in the central nervous system on the basis of behavioural and biochemical evidence obtained in whole animal studies; in addition, the drug stimulates adenylate cyclase in striatal homogenates with a potency comparable with that of dopamine. The effects of apomorphine (including those two presently studied) are, like those of dopamine, antagonised by antipsychotics¹³.

It is unlikely that pretreatment with haloperidol or apomorphine interfered with the metabolism of the alkaloid since such a mechanism would have resulted in similar shifts in responsiveness in the two tests, which was obviously not the case. In addition, chronic pretreatment with haloperidol did not modify the apomorphine distribution in the brain⁷. It is also unlikely that the diminished hypothermic response to apomorphine resulted from a nonspecific compensatory adaptation of thermoregulatory mechanisms to the initial hypothermia since the response of other hypothermia-inducing agents like oxotremorine, clonidine, or promethazine, was not modified in apomorphine-pretreated mice. Also, subsensitivity to apomorphine developed as well when the hypothermia produced by the first treatment was prevented by keeping the mice in a hot environment.

Several studies have recently indicated that dopamine receptors in the CNS mediating various behaviours are either supersensitised by pharmacological interruption of synaptic function^{4,7} or, conversely, other receptors may be desensitised by sustained stimulation⁸. These results were, however, obtained by treatments extending over periods lasting from several days to several weeks. The striking feature in our experiments, which could bring new light to the process involved, was the rapid onset of the state of modified receptor sensitivity following the administration of a single dose of either the agonist or the antagonist. In fact in both cases there was no apparent time lag between the end of the drug effect and the appearance of this state of modified sensitivity, suggesting that the modifications take place while the drug is still acting on the receptor.

These events could be explained by postulating a conformational or functional change in the receptor as a direct result of drug combination or, more probably (as it is also observed after denervation), as an indirect consequence of the altered impulse activity in the postsynaptic neurone. The altered receptor would have either a different affinity for the agonist or a modified ability to initiate the characteristic responses: the shift in the dose-response curves, without alteration of the maximal responses (especially in the case of hypothermia which is clearly a gradual process) rather favours the first hypothesis.

It is interesting that the state of modified sensitivity had approximately the same duration in the two cases (that is, after hypersensitisation by haloperidol or desensitisation by apomorphine) which suggests that similar recovery processes are involved. The rather long duration of these states of modified sensitivity to apomorphine, which outlast by several days the drug action, may represent the time required either to replace non-reversibly modified receptors by newly-synthesised ones or, alternatively, to stabilise compensatory neuronal circuits.

The other interesting feature of the present observation is the easy desensitisation of dopamine receptors involved in thermoregulation which contrasts with their inability to exhibit hypersensitivity, although the opposite characterised those mediating the climbing behaviour, which could be hypersensitised but not desensitised. Reasons for this difference are not clear at present, but may be related to their location in different brain areas. The receptors mediating hypothermia seem to be localised in the mesolimbic system¹³ or, possibly, in the newly discovered dopaminergic system in the hypothalamus¹⁴. On the other hand, the climbing behaviour, which was suppressed by electrocoagulation

of the striatum and facilitated after denervation by 6-hydroxydopamine injected into this region (P.P., J.C. and J.C.S., unpublished), seems to be mediated by striatal receptors.

The exact relationship between the opposite changes in sensitivity and the location in different brain areas remains to be explored, however. One possible explanation could be that the first category of dopamine receptors (mediating the hypothermia) are, before any treatment, already in a state of maximal functional sensitivity whereas the reverse is true for the others, a difference which could result from the different activities in the respective presynaptic neurones. Other cerebral receptors, mediating the activation of cyclic AMP synthesis elicited by noradrenaline could be either desensitised¹⁵ or hypersensitised¹⁶.

Whatever their mechanism, the rapid, sustained and independent changes in sensitivities of different dopamine receptors in the brain may have important implications. It could provide an additional way to differentiate classes of dopaminergic synapses, a task not yet feasible by observing the short-term effects of drugs. It could be helpful in the development of animal models of diseases associated with altered dopaminergic transmission in the CNS. Finally, it could possibly offer a new therapeutic approach to these diseases.

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Release of ATP from stimulated nerve electroplaque junctions

THREE successive phases can be distinguished when the amplitude of the electrophysiological response of *Torpedo* electroplaques is recorded during stimulation at 5 or 10 Hz (ref. 1). A decrease, lasting about 30 s, is followed by a plateau of about 30 s during which the amplitude is maintained at 30-40% of its initial value. After the plateau, a late decrease brings the response to negligible levels in a few minutes. These three phases are correlated with variations in the tissue level of total acetylcholine (ACh)—the amount of transmitter decreasing during the first phase, increasing to control levels during the plateau and declining again. These variations are about 30-40% of the total level of ACh. No change is observed when only the pool of bound ACh is measured^{2,3}. In *Torpedo* homogenates, bound ACh (protected from hydrolysis by esterases³) is associated with a population of synaptic vesicles, which have been isolated by fractionation techniques⁴. It has been shown that ATP is

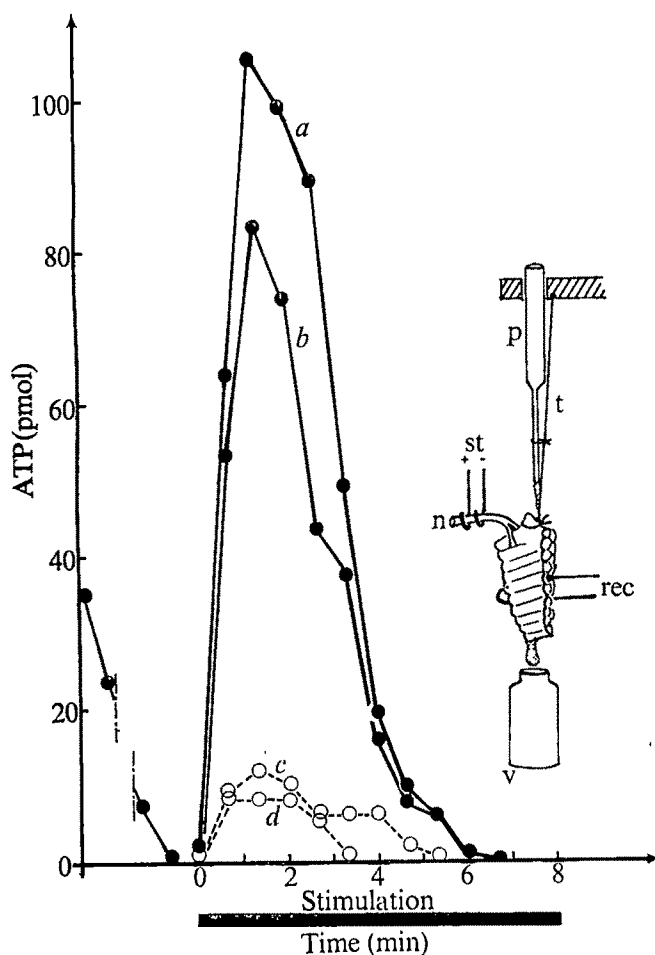


Fig. 1 Electric tissue and corresponding nerve. Perfusion fluid is brought by the Pasteur pipette (p), and the drops collected in the vial (V); a thread (t) holds the tissue; the nerve (n) is stimulated by the electrodes (St), and the response recorded by recording electrodes (Rec). Recording electrodes at each end of one prism. ●, Without curare; ○, with curare. *a* and *b*, Two experiments showing that ATP was collected in the perfusate when the nerve is stimulated. *c* and *d*, Curare inhibited the release of ATP. A section of electric organ (1 or 2 prisms thick) was dissected with its corresponding nerve, the fish being anaesthetised with Tricaine (1 g per 3 l seawater). The tissue was superfused with a physiological solution as described previously. The fluid (1 drop every 10 s) was collected in vials, in which the ATP assay was immediately carried out using the firefly luciferin-luciferase method. The Sigma-buffered firefly lantern extract was dissolved in 5 ml water and 100 μ l of the solution was added to the sample containing ATP. The light emission was measured using a liquid scintillation spectrometer SL30 intertechnique.

present in the vesicular fraction⁵. When *Torpedo* electric organ tissue is stimulated more than would be necessary to exhaust the electrophysiological response, or when for some other reason synthesis of new transmitter is rendered inefficient, a decrease in bound or vesicular ACh is observed. Of all the variations in ACh described, only the decline in bound ACh has been thoroughly investigated and confirmed by other laboratories⁶. It is known from fractionation experiments that both ATP and ACh are associated with synaptic vesicles, and a decrease in the vesicular content of the two substances was found in the experimental conditions that produce a decline in the pool of bound ACh (ref. 6). Several authors have collected ATP in the perfusate from stimulated neuromuscular junctions⁷⁻⁹, but the nucleotide was not found in perfusates from stimulated autonomic ganglia¹⁰.

As the electric organ of *Torpedo* is richly innervated and homologous to neuromuscular junctions, the preparation may be used to determine whether or not the nucleotide is

released, and if so from what structure such release occurs (for details of method, see Fig. 1).

Figure 1 shows that some ATP is released when the tissue is mounted, but this rapidly decreases to background levels. The stimulation (pulses of 1 ms duration at 5 Hz) was delivered to the nerve. A maximal initial response of 6–8 V was recorded and its decline followed during a 10-min stimulation period. Immediately after the onset of stimulation, ATP appears in the superfusate and its level falls when the electrophysiological response is exhausted. (Fig. 1*a* and *b*). In a similar experiment, carried out after incubating the tissue for 30–60 min in *d*-tubocurarine chloride 10^{-4} M, the electrophysiological response was reduced to a few 100 mV and the amount of ATP released on stimulation became negligible (Fig. 1*c* and *d*). In similar experimental conditions using labelled precursors of ACh, we have shown that the release of transmitter was not affected by curare. If we assume that the only effect of curare is to protect the post-synaptic cell from the effects of ACh, these results suggest that ATP release during stimulation is a postsynaptic event. In previous work, it has also been shown that the tissue level of ATP declines during stimulation and that this effect is inhibited by curare¹¹.

Such ATP release may be considered as a postsynaptic event probably independent of the decrease in vesicular ATP occurring when the bound pool is utilised. We have also observed^{12,13} in-phase oscillations in the levels of ACh and ATP during stimulation. These fast oscillations (5-s period) are still not fully understood and may not necessarily be connected with the release of ACh from the nerve terminal or with postsynaptic ATP release. Moreover, it has previously been observed that addition of ATP to the incubation bath modifies the evolution of the electrophysiological response during stimulation (transient increase of the plateau)¹⁴. All these observations indicate clearly that ATP exerts its action at different levels during synaptic activity.

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Voltage clamp study on inward chloride currents of spherical muscle cells in tissue culture

EMBRYONIC chick skeletal muscle fibres grown in tissue culture generate three types of action potential different in their underlying ionic mechanisms and which are known accordingly as Na, Ca and Cl spikes¹. Because of their irregular and cylindrical shape^{2,3} these muscle fibres are not suitable for further analysis of the ionic mechanisms in using voltage clamp techniques. Voltage clamp techniques could however be applied successfully to those cultured muscle cells which, after incubation in a medium containing colchicine, had become spherical

myosacs²⁻⁶. In preliminary studies, the myosac generated Na, Ca and Cl spike components² and also, under voltage clamp, a late inward current (LIC) not necessarily due either to Na or to Ca (ref. 3). I now report a further voltage clamp study of the myosac revealed that the amplitude of the LIC was related to the concentration gradient of Cl ions across the cell membrane, and that the LIC was generated by a slow increase of Cl conductance which was dependent not only on the membrane potential, but also on the time course of the membrane depolarisation. Such Cl conductance provides the basis for the generation of a regenerative Cl spike in tissue-cultured skeletal muscle cells.

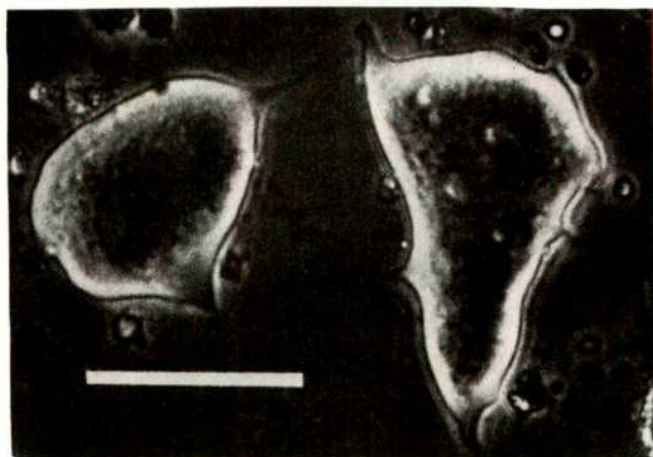


Fig. 1 Two myosacs on the 7th day in tissue culture. 10^{-8} M colchicine was added to the tissue culture medium during the 4th and 6th day of muscle development. Phase contrast microscope. Scale: 50 μ m.

Myoblasts (3×10^5 to 4×10^5 cells ml^{-1}) were obtained from pectoral muscles (dissociated mechanically) of 10–12-d old chick embryos and grown on a collagen-coated plastic dish (Falcon) in a modified Eagle's minimum essential medium⁷. Colchicine (10^{-8} M final concentration) was added to the medium during the 4th and 6th day of tissue culture, and the original long multinuclear myotubulus developed into independent myosacs 10–200 μ m in diameter (Fig. 1).

A myosac was penetrated with two glass microelectrodes (8 to 15 M Ω) filled with either 3 M KCl or 3 M K acetate solution. Membrane currents were measured with conventional voltage clamp techniques^{3,8}. To prevent possible inactivation of spike generation at low resting potentials² (-50 to -60 mV), the myosac was kept hyperpolarised to between -70 and -100 mV (holding membrane potential) by injecting steady currents of 2 to 10 nA (holding current). This holding current was also used for an injection of anions contained in the microelectrode into the myosac. The ionic composition in the bathing solution (NaCl 128.4 mM, KCl 5.0 mM, CaCl_2 5.0 mM and Tris-Cl 15.0 mM at pH 7.4) were altered under the voltage clamp condition.

Figure 2a–f illustrates the membrane currents generated by stepwise changes of the intracellular potential from the holding potential (-90 mV) to various levels. In this experiment, the microelectrodes were filled with 3 M K acetate solution. For small ranges of depolarisation or hyperpolarisation, the membrane currents consist of a transient capacitive current which lasts for a few ms (not visible in the figure) and a steady leakage current (Fig. 2e and f). When the membrane depolarisation exceeds a certain level (threshold), a LIC is seen superimposed on the two currents above. The LIC reaches its maximum amplitude of 2–5 nA in 1 to 10 s after depolarisation and decays thereafter to a level of the leakage current (Fig. 2d); the generation of the LIC is apparently time dependent. The same LICs can be generated repeatedly if the interval between the depolarisation is sufficiently long (usually 60–100 s). By increasing the depolarisation (Fig. 2d–a), the LIC becomes smaller in ampli-

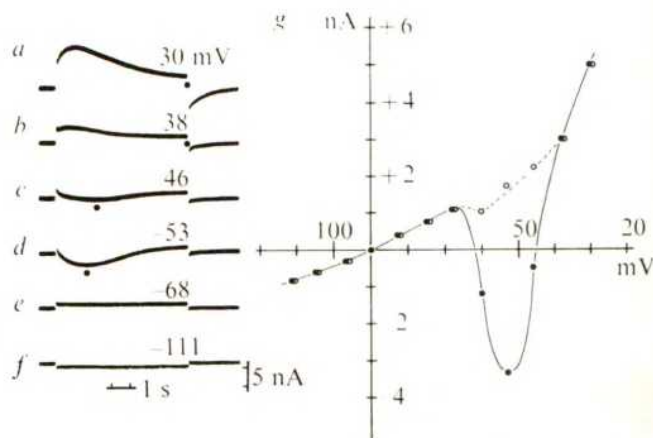


Fig. 2 Membrane currents and the I–V relationship examined under voltage clamp. a–f, Membrane currents elicited by instantaneous changes of the intracellular potential from the holding potential (-90 mV; holding current, 3.5 nA) to the levels indicated on each records. LIC is seen maximum amplitude in d. ●, Amplitudes of the peak inward currents or of the least outward currents (marked below records) during the depolarisation. ○, Amplitude of the currents measured at the end of the depolarisation. K acetate-filled microelectrodes were inserted into a myosac on the 5th day in tissue culture. See text for further details.

tude (Fig. 2c) and finally, most of the membrane currents become outward during a large depolarisation (Fig. 2a and b). It should be noted that shortly after the depolarisation, the myosac generated a transient inward current (50 to 200 nA) lasting 1–5 ms; this inward current, which was shown to be carried by Na ions³, is not seen in the figures of this paper because of the slow sweep velocity.

Figure 2g is an I–V relationship of the membrane currents obtained by plotting (1) the peak inward currents or the least outward currents and (2) the currents measured at the end of the depolarisation of Fig. 2a–g against the membrane potentials. The plots for the peak of the LICs form the N-shaped curve in Fig. 2g. This indicates that the LICs are generated as a result of an increase in the membrane conductance which represents the negative resistant part of the I–V curve and that this conductance increase is depended on the membrane potentials.

The LIC in Fig. 2 reaches its maximum amplitude between -50 and -60 mV. The maximum LIC was tested in the following ways. When Cl ions were injected into a myosac by means of a nearly steady current (holding current; see above) through a KCl-filled microelectrode, the amplitude of the LIC increased

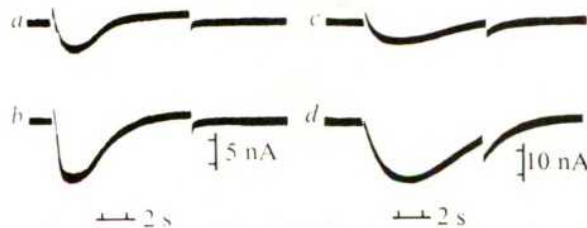


Fig. 3 Increase in amplitude of LICs associated with changes of Cl ion concentration. a–b, Intracellular injection of Cl ions by holding membrane current (about 4 nA) through a KCl-filled microelectrode caused a gradual increase in the amplitude of LICs elicited by the membrane depolarisation from -85 mV to -55 mV. a and b were recorded 5 and 11 min after the onset of the Cl injection, respectively. A myosac on the 6th day in tissue culture. c–d, Decrease of the extracellular Cl ion concentration (replaced by acetate ions) from 158.4 mM (c) to 15.0 mM (d) caused the increase in the amplitude of the LICs elicited by the membrane depolarisation from -75 mV to -56 mV. Before recording c, the myosac (on the 7th d in tissue culture) was loaded with Cl ions by 2 to 3 nA holding current for about 15 min, and the LICs became steady in amplitude. The solution change from c to d was performed in 3 min.

in time. This is shown in Fig. 3*a* and *b* by comparing the LIC elicited at about 11 min (*b*) with that at 5 min (*a*) after the onset of the Cl injection. In spite of the increase in amplitude, the time course of the membrane currents remained almost constant during the injection of Cl ions (Fig. 3*a* and *b*). Such an increase in amplitude of the LICs, however, did not occur (or rather decreased slightly) in the control experiments where acetate ions, instead of Cl ions, were injected intracellularly through a K acetate-filled microelectrode (not illustrated). Since acetate ions are considered impermeable through various Cl-permeable membranes⁹⁻¹¹, the increase in the amplitude of the LIC described above could be attributed to an elevation of Cl ion concentration in the intracellular space of the myosac.

The amplitude of the LIC became almost steady after about 10–15 min of the Cl-loading procedure. When Cl ion concentration in the bathing solution was decreased by replacing Cl ions with an equimolar solution of acetate (or propionate) ions, the LICs increased further in amplitude (Fig. 3*c–d*). But an alteration of the cation species in the bathing solution, such as Na (replaced by Tris), K (replaced by Na in the range 2–20 mM) or Ca (replaced by Na in the range 2–20 mM) ions, did not induce a significant increase in the amplitude of the LICs (not illustrated). These observations indicate that the amplitude of the LIC is related to both the intracellular and extracellular concentration of Cl ions, namely the concentration gradient of Cl ions across the myosac membrane.

It should be pointed out that the LICs were elicited under conditions in which the membrane potential was held at a hyperpolarised level below its resting membrane potential and the myosac was loaded with Cl ions. In these conditions, the electromotive force of the LICs, E_{Cl} , would be more positive than the holding membrane potential. An increase in the Cl conductance at the membrane potential depolarised below E_{Cl} would therefore generate an inward current³. It should also be mentioned that the net flux of the Cl ions during a LIC was directed outward.

The slow time course (that is, the peak time and duration) of the present LICs obtained in the voltage clamp experiments is compatible with the slow rise time and long duration of the Cl spikes elicited in the myosac under a current clamp condition. In fact, when a myosac was examined under the voltage clamp and current clamp conditions, the reversal potential of the LIC coincided closely to the peak membrane potential of the Cl spike³. These observations support the idea that the Cl conductance underlying the LIC is responsible for generation of the Cl spikes in cultured skeletal muscle fibres.

Inward Cl currents generated in *Nitella* and *Chara*¹²⁻¹⁴, and in electric organs of fish¹⁵ resemble the present LIC with respect to the time course and the dependency on the membrane potentials. Although the direction of currents is outward, a similar slow increase in Cl conductance has been reported in mature frog skeletal muscles^{16,17} and in sheep heart muscles¹⁸. The physiological role of the increase in the Cl conductance is not known for certain. Cl conductance may have a role in the coupling between the excitation and contraction¹⁹⁻²¹ or in the differentiation and maturation of muscle cells.

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Ionic permeability changes occurring at excitatory receptor membranes of chemical synapses

ALTHOUGH the ionic basis for generation of action potentials in nerve and muscle cells is now well understood¹, there is still much uncertainty about the changes in ionic permeability which result in the generation of graded depolarisations at receptor membranes of excitatory chemical synapses². The equilibrium potential for these graded depolarisations (E_R) is usually between -20 and $+10$ mV. This suggests an underlying increase in sodium permeability (P_{Na}) and potassium (P_K) and/or chloride (P_{Cl}) permeability, for in most nerve and muscle cells, the sodium equilibrium potential (E_{Na}) is very positive ($+50$ to $+75$ mV) and the potassium and chloride equilibrium potentials (E_K and E_{Cl}) are very negative (-40 to -100 mV). There is evidence that an increase in P_{Na} does occur during activation of excitatory synaptic membranes³, but there are few such membranes for which there is evidence for an increase in either P_K or P_{Cl} . Here we review and analyse the data obtained from previous investigations on the ionic basis of the graded depolarisations occurring at excitatory synapses. We demonstrate that the application of the Goldman-Hodgkin-Katz equation³⁻⁴ to previous data suggests that an increase in P_K as well as P_{Na} probably occurs at most if not all excitatory synapses.

At the vertebrate endplate, the transmitter substance and acetylcholine cause an increase in sodium and potassium conductance (Δg_{Na} and Δg_K), and

$$\frac{\Delta g_{Na}}{\Delta g_K} = \frac{E_K - E_R}{E_R - E_{Na}} \quad (1)$$

$\Delta g_{Na}/\Delta g_K$ remained constant during changes in external sodium concentration $[Na]_o$ and external potassium concentration $[K]_o$ between 5–150 mM and 0.25–7 mM respectively^{5,6}, with tenfold changes in $[Na]_o$ and $[K]_o$ within these concentration ranges causing shifts of E_R of about 30 and 25 mV respectively^{5,6} (Figs 1 and 2). Similar results were obtained at two other nicotinic cholinergic excitatory synapses, namely synapses generating the fast excitatory postsynaptic potentials in the frog sympathetic ganglion⁷, and the annelid neuromuscular junction⁸ (Figs 1 and 2).

For many other synapses, however, such as several cholinergic synapses in the molluscan central nervous system⁹⁻¹¹, glutamate synapses of insect^{12,13} and crustacean¹⁴ excitatory neuromuscular junctions, and mammalian smooth muscle cholinergic junctions¹⁵, E_R was altered by changes in $[Na]_o$, but not by changes in $[K]_o$ or $[Cl]_o$ (Figs 1 and 2). For example, in voltage clamp studies of the insect neuromuscular junction¹², E_R for the glutamate current and the excitatory postsynaptic current was $+3$ and $+4$ mV respectively in normal saline. In 0% NaCl, E_R for the glutamate current was $+8$ mV. E_R was not, however, altered when $[Cl]_o$ was decreased by 100% or when $[K]_o$ was changed between 0 and 20 mM. Consequently, it has been suggested that the synaptic current at all of

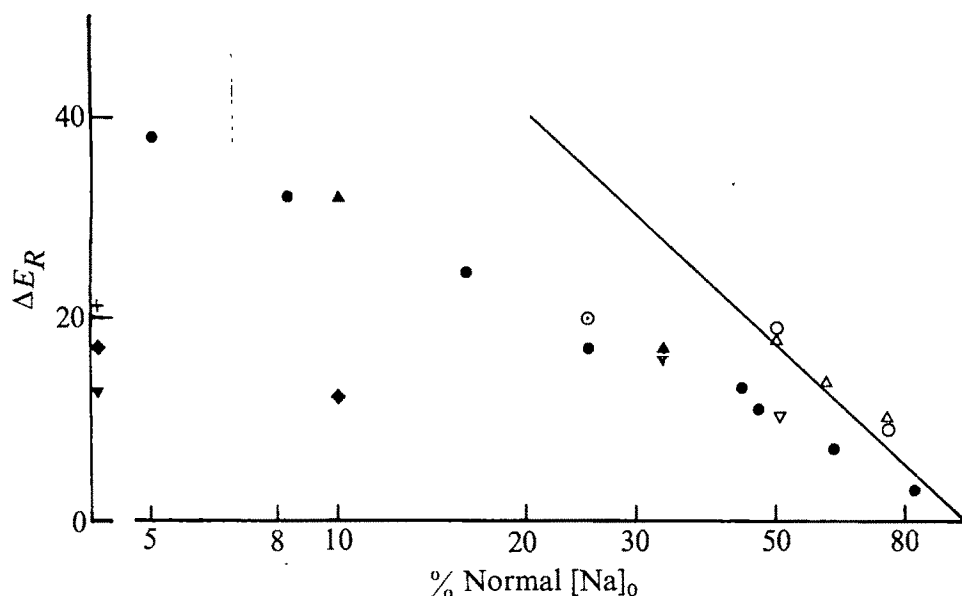


Fig. 1 Equilibrium potential (E_R) of the synaptic potentials and currents at different sites in different external concentrations of sodium, $[Na]_o$. The line represents a slope of 58 mV per tenfold change in $[Na]_o$. Most of the values of E_R are below both this 58 mV slope predicted by the Nernst equation if just Na was carrying the synaptic current, and also below the 50–57 mV slope predicted by the Goldman-Hodgkin-Katz equation if both Na and K were carrying the synaptic current (see Fig. 3). Abscissa: $[Na]_o$ (log scale). Ordinate: change in E_R . The data have been obtained from the following sources: ●^{6,8}, ○¹¹, △¹⁰, ▽¹⁴, ▽⁷, ○⁸, +⁸, ◆¹², ▲¹¹, ■¹³.

these sites was probably due exclusively to Na activation⁹⁻¹⁵. There are also several other excitatory synaptic membranes at which it has been concluded that only Na carries the current during activation (for review see refs 2 and 16).

The 'membrane conductance' of an ion depends not only on the membrane permeability for that ion but also on the concentration and distribution of that ion on either side and within the membrane¹. Changes in $[Na]_o$ and $[K]_o$ would therefore be expected to affect the membrane conductance but not the membrane permeability of these ions. We have used the Goldman-Hodgkin-Katz equation to predict changes in E_R when $[Na]_o$, $[K]_o$ and $[Cl]_o$ are altered at the crustacean neuromuscular junction, the molluscan central nervous system and the squid giant synapse, using the data of previous investigators^{10,14,17}. These sites were chosen as they illustrate

in E_R predicted for a constant permeability increase to Na and K are very different from those predicted for a constant conductance increase to Na and K, but they do agree closely with the observed results at many excitatory synapses. We therefore propose that an increase in conductance to both Na and K does occur at excitatory synapses at which E_R lies between E_{Na} and E_K and at which altering $[K]_o$ between 0 and about 200% normal does not cause an observable shift in E_R . At the vertebrate endplate, E_R for the endplate potential at low temperatures (2 °C), and E_R for the action of suberythrolone, a cholinomimetic agonist, are not changed when $[K]_o$ is lowered from 2.5 to 0.5 mM (ref. 18). It was therefore suggested that $\Delta P_{Na}/\Delta P_K$ does stay constant for these conditions when $[K]_o$ is lowered¹⁸.

Although there is much evidence that Na activation occurs

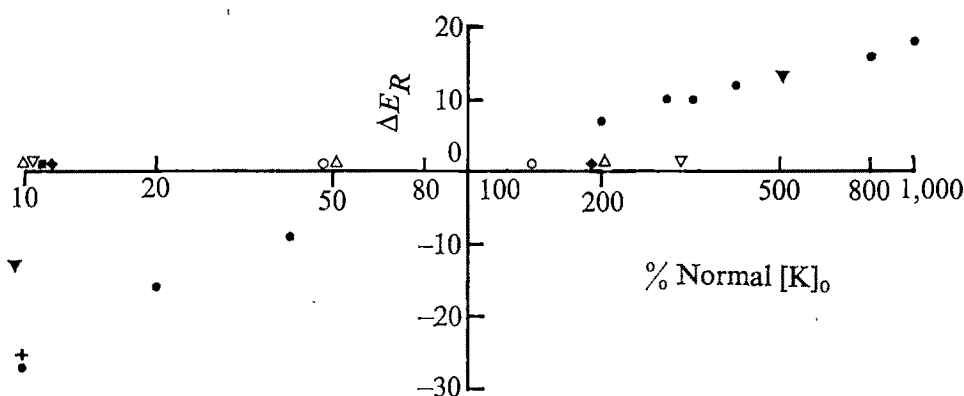


Fig. 2 Equilibrium potential (E_R) of the synaptic potentials and currents at different sites in different external concentrations of potassium, $[K]_o$. Abscissae, $[K]_o$ (log scale). Ordinates, change in E_R . References for symbols as in Fig. 1.

the predictions of the Goldman equation for a large range of permeability ratios and ionic concentrations. The analysis was carried out assuming that a permeability increase occurred to either Na and K (ΔP_{Na} and ΔP_K) or Na and Cl (ΔP_{Na} and ΔP_{Cl}). The values of $\Delta P_{Na}/\Delta P_K$ were calculated in normal saline from the equation:

$$E_R = \frac{RT}{F} \ln \frac{[K]_o + \Delta P_{Na}/\Delta P_K [Na]_o}{[K]_i + \Delta P_{Na}/\Delta P_K [Na]_i} \quad (2)$$

To calculate $\Delta P_{Na}/\Delta P_{Cl}$, the terms for K were replaced by those for Cl. $\Delta P_{Na}/\Delta P_K$ and $\Delta P_{Na}/\Delta P_{Cl}$ were then assumed to stay constant when $[Na]_o$, $[K]_o$ and $[Cl]_o$ were altered. It can be seen from Fig. 3 that the equation predicts that varying $[Na]_o$ and $[Cl]_o$ causes large changes in E_R , whereas varying $[K]_o$ causes little change in E_R . In fact, the theoretical curve for log $[K]_o$ against E_R has a maximum slope of only about 2 mV for $[K]_o$ between 0 and 200% normal. These changes

at the majority of chemical excitatory synapses, the changes in E_R caused by lowering $[Na]_o$ are often much less than those predicted by the Goldman-Hodgkin-Katz equation if Na and K activation occurred (Fig. 1). A possible explanation is that calcium, which usually has a very positive equilibrium potential, is carrying some of the inward current. There is evidence that the synaptic membrane at the vertebrate endplate¹⁹ and at the insect¹² and crustacean^{14,20} neuromuscular junction is slightly permeable to calcium. Alternatively, lowering $[Na]_o$ may cause a reduction in ΔP_K (ref. 20 and R.A. and P.N.R.U., unpublished).

In conclusion we believe that the graded depolarisations recorded at excitatory synapses which involve an increase in conductance normally involves an increase in both P_{Na} and P_K . In addition, an increase in P_{Ca} may occur especially in low $[Na]_o$. The sites that have been examined in sufficient detail can be divided into two categories. In the first category, which seems to include the nicotinic ACh synapses at the frog

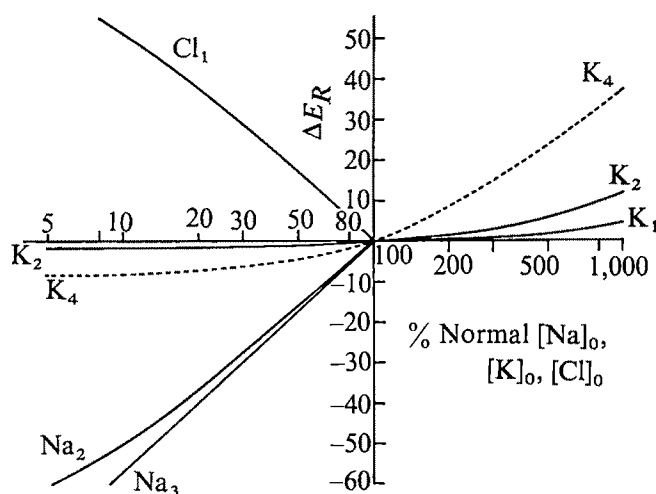


Fig. 3 Equilibrium potential (E_R) of the synaptic currents in different external concentrations of Na, K and Cl, as predicted by the Goldman-Hodgkin-Katz equation. The concentrations of ions and E_R in normal saline were taken from the data of previous investigations. (1) Crustacean neuromuscular junction¹⁴. (2) Molluscan central nervous system¹⁰. (3) Squid giant synapse¹⁷. (4) Crustacean neuromuscular junction (10 mM Na). The curves for Na_1 lie between Na_2 and Na_3 ; K_3 lies very close to the abscissae, and Cl_2 and Cl_3 are very close to Cl_1 . It can be seen that altering $[Na]_o$ and $[Cl]_o$ causes large changes in E_R , whereas altering $[K]_o$ causes little change in E_R , especially between 0 and 200% normal $[K]_o$, the range of concentration used by most investigators. In very low Na (10 mM), altering $[K]_o$ does cause much larger changes in E_R . The slope of the line relating $\log [Na]_o$ to E_R between 50% and 100% $[Na]_o$ is very similar for both the squid giant synapse and the molluscan CNS (57 mV and 52 mV respectively) in spite of $\Delta P_{Na}/\Delta P_K$ being 17.0 at the former and 0.3 at the latter.

and annelid neuromuscular junction and the frog sympathetic ganglion, the increase in conductance ratio to Na and K stays constant when the external ionic environment is modified. In the second category, which includes the acetylcholine synapses in the molluscan central nervous system, insect and crustacean glutamate synapses, and also probably the muscarinic acetylcholine synapses on mammalian smooth muscle, the increase in the ratio of Na conductance to K conductance changes when the external ionic environment is lowered, although the increase of the ratio of Na permeability to K permeability probably stays constant, especially during changes in $[K]_o$.

We would like to thank Dr P. C. Caldwell and Dr B. L. Ginsborg for reading a draft version of this manuscript. This work was supported by a grant from the SRC to P.N.R.U. *Note added in proof:* Ritchie and Fambrough²¹ have found that the acetylcholine reversal potential of cultured rat myotubes can be described by the Goldman equation in media of different $[K]_o$.

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Chemical modification of crab nerves can make them insensitive to the local anaesthetics tetrodotoxin and saxitoxin

CONDUCTION of the nervous impulse depends on voltage-sensitive change in the permeability of the nerve cell membrane to sodium ions. The sodium channels that appear during the action potential can be blocked very specifically by various neurotoxins, including tetrodotoxin (TTX) from the puffer fish and saxitoxin (STX) from certain marine algae¹. Shrager and Profera² presented evidence that the binding of TTX to crab nerves is reduced after treatment with a water-soluble carbodiimide and glycine methyl ester as the associated nucleophile. In these chemically modified nerves it follows that the sodium channels should have been rendered insensitive to TTX. Shrager and Profera were unable to test this prediction because in their experiments the nerves became inexcitable after chemical treatment.

We have carried out a similar modification and found conditions in which nerves continue to conduct impulses. Axons treated in such a way have a greatly reduced sensitivity to both TTX and STX, suggesting that either the sodium channel or its immediate environment has been modified such that the binding of both TTX and STX is markedly impaired without appreciably affecting the access of ions to the voltage-sensitive sodium channel.

In all our experiments, a water-soluble carbodiimide was used with various associated nucleophiles. Nucleophiles with pK_s in the neutral range and below were most satisfactory and these included diglycine, triglycine, glycine methyl ester, glycine ethyl ester, imidazole, semicarbazide, orthophosphate and *p*-amino benzene sulphonate.

Nerves were obtained from the walking legs of the spider crab (*Maia squinado*) and exposed to reagents in artificial seawater containing NaCl, 460 mM; MgCl₂, 55 mM; CaCl₂, 11 mM; KCl, 10 mM; NaHCO₃, 2.5 mM; together with 100 mM nucleophile and 100 mM carbodiimide at pH 5.5. After varying lengths of time the nerves were removed, washed well in normal artificial seawater (pH 7.8), and their sensitivity to TTX examined. In many instances treated nerves continued to conduct action potentials in the presence of 0.1 mM TTX, whereas untreated control nerves were blocked by TTX concentrations as low as 50-100 nM. To quantify this observation we used the conduction velocity technique of Colquhoun and Ritchie³. Nerves were mounted on a spaced set of platinum electrodes, and after the reaction, the conduction velocity was measured as a function of the external sodium concentration. In this way it is possible to equate inhibition produced by TTX to an equivalent reduction in external sodium and to obtain a rough estimate of the number of sodium channels that have been blocked, assuming $n=1$ in equation (3) of Colquhoun and Ritchie (Fig. 1).

Two features should be noted. The dose-response curve seems to be made up of two components: one with a normal high affinity for TTX, which presumably reflects unreacted sodium channels, and one with an affinity three

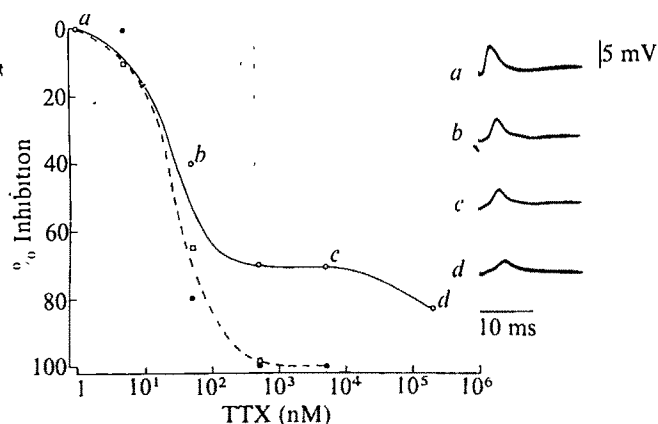


Fig. 1 Dose-response curves for blockage by TTX of sodium channels in crab nerve. Ordinate, reduction in number of functional sodium channels plotted as percentage inhibition; abscissa, concentration of TTX (nM) plotted on a log scale. ●, Control nerve immersed in artificial seawater (ASW); ○, nerve pretreated for 30 min in ASW containing 100 mM glycine methyl ester and 100 mM carbodiimide, pH 5.5 at 21 °C, compound action potentials corresponding to points *a*, *b*, *c*, *d* are shown on the right; □, nerve exposed to previous solution but in presence throughout of TTX (500 nM). After reaction, nerves were washed well in ASW before determining sensitivity to TTX. All nerves were obtained from the same animal.

to four orders of magnitude lower. These modified sites are partly inhibited in the presence of a saturated solution of TTX, but it is possible that at these concentrations TTX or an impurity is acting in a nonspecific manner. Exposure to carbodiimide and a suitable nucleophile while in the presence of a lethal concentration of TTX followed by repeated washing in TTX-free medium restored a conducted action potential that exhibits a sensitivity to TTX comparable with that seen in untreated controls. This observation shows that the presence of TTX protects against formation of the insensitive (or less sensitive) TTX-binding site.

To increase the proportion of sodium channels protected, we carried out the reaction in various conditions. As expected from the known chemistry of the carbodiimide reaction⁴ (Fig. 2), the rate of reaction increases as pH is reduced; but at low pH the nerve suffers irreversible damage even in the absence of reagents. We normally worked at pH 5.3–5.8. In these conditions the reaction rate increased linearly with increasing concentrations of either carbodiimide or glycine methyl ester. Some damage, evidenced by a reduction in conduction velocity, was seen in all preparations after exposure to the carbodiimide either alone or with a nucleophile. Glycine methyl ester alone had no effect on the preparation. Prolonged exposure to carbodiimide eventually blocked conduction, and the onset of this conduction block provided an upper limit to the exposure time. In optimum conditions (100 mM glycine methyl ester, 100 mM carbodiimide pH 5.5 for 30 min) we obtained modification of about 40% of the functional TTX-binding sites. The same treatment rendered a comparable percentage of sodium channels insensitive to another local anaesthetic, STX.

What group in the membrane is involved in generating the observed sensitivity to TTX? In aqueous solution the main groups known to react in the conditions of our experiments are thiols, phenols, amines, phosphates and carboxylates. In general, three reaction schemes are possible (Fig. 2.).

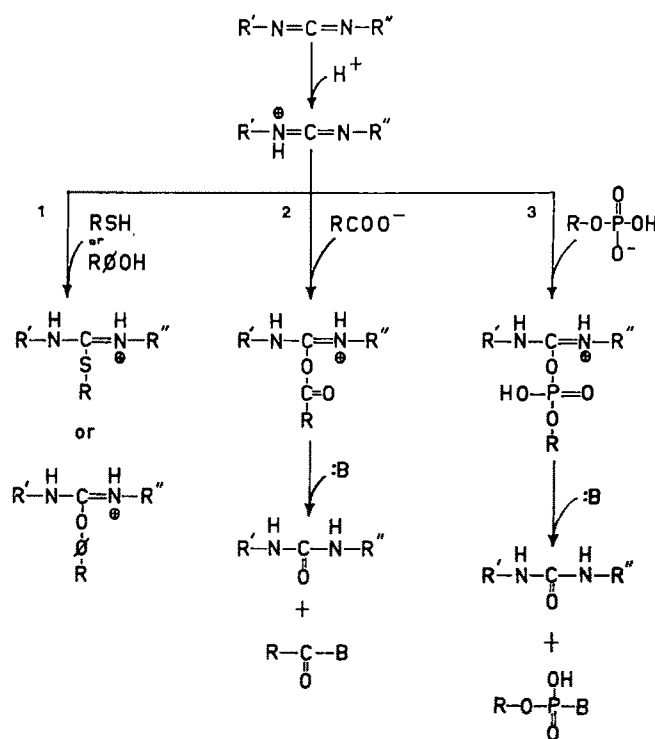
(1) The reactions with thiols, phenols and amines yield stable products without any added nucleophile³. It follows that reaction with carbodiimide alone should lead to a reduction in sensitivity to TTX, but this is never seen. Nerves exposed to carbodiimide alone slowly become in-

excitable, which may result from these reactions, or, conceivably, from reaction with a surface phosphate group either by effecting cyclisation of a phosphate with an adjacent *cis* hydroxyl or formation of a N-phosphorylurea^{4,6}. (2) The carbodiimide reaction with carboxyl groups yields an unstable intermediate which will react with a nucleophile. When the nucleophile is a primary amino compound the reaction should give a stable amide bond. Condensates with secondary amino compounds will be less stable. If, however, the nucleophile is orthophosphate, the product should be an acylphosphate which would be expected to hydrolyse at pH 5.5 with a halftime of less than one hour⁷. (3) With membrane phosphate groups, condensation with primary and secondary amines will form phosphoramidates; condensation with carboxyl will form acylphosphate; and condensation with orthophosphate will form pyro- or polyphosphates. Although the phosphoramidates are stable at pH 7 (refs 8–11), both acyl- and pyrophosphate are relatively easily hydrolysed^{7,12,13}.

There are problems in distinguishing experimentally between membrane-bound phosphate and carboxylate. A short-lived reduction in sensitivity to TTX (30 min) was seen after condensation with orthophosphate, which is consistent with both possibilities. In theory these possibilities might be distinguished if protection could be demonstrated after reaction with a carboxylic acid as nucleophile, because anhydrides are generally much less stable in aqueous solution than acylphosphates¹⁴. Condensation with carboxylic acids normally block conduction rapidly; but some protection against TTX was obtained with malonic acid, suggesting that a phosphate group may be involved. In addition, although not yet allowing further discrimination, we find that reaction with Meerwein's reagent (triethyloxonium tetrafluoroborate) also renders nerves insensitive to TTX.

The observation that TTX is able to protect against the chemical modification produced by carbodiimide and a suitable nucleophile raises the possibility that this might provide a convenient means of attaching a covalent label to the sodium channel. Thus, if a nerve is exposed to

Fig. 2 Possible reaction schemes involving membrane components, carbodiimide and nucleophiles. R, Unknown component of the membrane; R', ethyl; R'', dimethylaminopropyl; B, various nucleophiles.



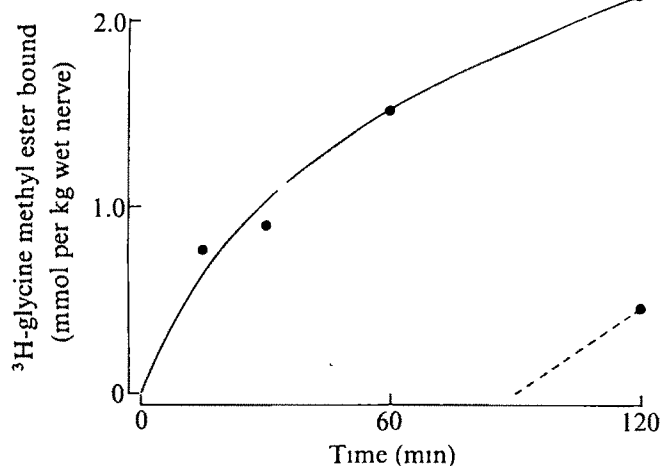


Fig. 3 Binding of ³H-glycine methyl ester to crab nerve. Nerves were exposed to ASW containing 100 mM carbodiimide and 100 mM glycine methyl ester at pH 5.5. After incubation, nerves were washed well in ice-cold ASW containing non-radioactive glycine methyl ester, homogenised in distilled water to release any free intracellular label and centrifuged. The pellet was washed twice with distilled water containing 10 mM ester, dissolved in Nuclear Chicago Solvent and counted. Temperature 21 °C. Uptake shown by dotted line was obtained after exposure of the nerve for 90 min to non-radioactive reaction mixture.

carbodiimide and glycine methyl ester in the presence of TTX, subsequent removal of the TTX and re-exposure to carbodiimide and labelled glycine methyl ester might result in specific labelling of the sites previously protected by the TTX. We tested this possibility by making the methyl ester of ³H-glycine using Fischer's method¹⁵, and measuring the uptake of label after reaction with crab nerve in optimal conditions. Figure 3 shows that uptake continued for many hours and that considerable radioactivity became associated with the nerve. If the uptake is expressed in terms of the available nerve membrane area¹⁵, after 60 min the uptake is equivalent to 10¹⁴ labelled molecules per cm² or 10⁶ per μm² or 1 molecule per 100 Å². The number of sodium channels has been estimated to be about 100 per μm² (refs 1, 17, and 18) and it is perhaps not surprising that we were unable to detect any significant differences in the uptake of ³H-glycine methyl ester between nerves pre-exposed to cold nucleophile in the presence or absence of TTX.

Labelled nerves provided further information on the nature of the covalent bonding. The majority of the label was stable to acid (1 h, pH 3.0), alkali (1 h, pH 10) and to neuraminidase; but was largely solubilised following exposure to a number of proteolytic enzymes including Pronase (Sigma Protease, Type V), trypsin and chymotrypsin, indicating that the label is mainly attached to proteins and polypeptides. The high density of these groups is somewhat unexpected although there is electrophysiological evidence for a similar number of negatively charged groups at the inner face of the squid axon membrane.

Our results show that a relatively straightforward chemical modification of the axon can lead to a dramatic change in its sensitivity to TTX and STX without affecting its voltage-sensitive permeability to sodium ions. This may help explain the relative insensitivity of some excitable cells to these toxins¹⁹. In its present form the carbodiimide-labelling technique produces too much nonspecific label to be of real use in isolating sodium channels, but it is probable that if the technique outlined here, or some modification of it, were applied to a partially purified preparation of TTX-binding sites it may be possible to attach a covalent label to the sodium channels that would aid purification of

these channels while still retaining their characteristic voltage-sensitive permeability to sodium ions.

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Direct evidence of importance of lysosomes in degradation of intracellular proteins

THE mechanisms of breakdown of intracellular proteins are largely unknown. It is widely presumed that lysosomes are important agents of this process¹, chiefly because they contain suitable enzymes². Although autophagic uptake and degradation of cytoplasmic organelles by lysosomes has been described¹, there is only circumstantial evidence for significant lysosomal involvement in turnover of intracellular proteins³⁻⁶. Indeed, it is difficult to explain the heterogeneous turnover rates of proteins⁷ by a process involving bulk uptake of material¹. Furthermore, there is evidence for alternate systems of intracellular protein breakdown^{5,8}. In contrast, it has been shown that macrophage lysosomes are largely responsible for the degradation of endocytosed proteins, and that cathepsin D participates⁹. In the work reported here, breakdown of endogenous proteins of the perfused rat liver has been retarded by a specific inhibitor of cathepsin D, directed to the lysosomes, thus demonstrating their importance in the process.

The pentapeptide pepstatin¹⁰ is an inhibitor of carboxyl proteinases (termed acid proteinases by Hartley¹¹, because they generally have acid pH optima) such as cathepsin D¹². Cathepsin D, a lysosomal² enzyme with optimum activity near pH 5, adapted to the probably acidic lysosomal milieu, is the only known carboxyl proteinase in rat liver. Data on neutral proteinases^{1,3}, which might be capable of proteolysis in the cytoplasm, continue to appear. But with the exception of the specific kidney endopeptidase renin (which is now known to be a carboxyl proteinase¹³), none of these enzymes is inhibited by pepstatin. Thus pepstatin has no effect on autoprolysis of rat liver cytosol proteins at neutral pH⁶. The time course (up to 4 h) of digestion of a cytosol protein mixture⁶ at neutral pH (where cathepsin D is inactive) by sonicated rat liver homogenate (5 mg protein per ml) in the presence of 1% Triton X-100 has now also been found to be unchanged by pepstatin (0.1-10 μg ml⁻¹). Pepstatin is therefore suitable for the specific inhibition of rat liver cathepsin D.

Table 1 Rate of output of TCA-soluble radioactivity

Addition made at 105 min	Rate of output	
	% Initial rate	Average of group (% initial rate \pm s.d. (n))
(a) None	97	100 \pm 4 (3)
	105	
	99	
(b) 2 ml 5 mM potassium phosphate buffer, pH 7.0, containing pepstatin (50 μ g ml ⁻¹)	107	101 \pm 6 (3)
	95	
	101	
(c) 2 ml control liposomes	81	93 \pm 13 (3)
	108	
	90	
(d) 2 ml liposomes containing pepstatin (50 μ g ml ⁻¹)	32	47 \pm 17 (3)
	66	
	44	

Perfusions and additions were as described for Fig. 1. Rats were 250–300 g body weight. Initial rates of output were between 2.6 and 4.7 d.p.m. per ml medium per min, and corresponded to the release of 2–3% of initial liver protein radioactivity per hour. Correlation coefficients for the lines for perfusions in the absence of liposomes or in the presence of control liposomes were between 0.94 and 0.99. Those for periods after treatment with liposomes containing pepstatin were lower (0.87–0.94), probably because inhibition was not immediate. Leakage of malate dehydrogenase during the perfusion was always less than 3%, confirming the integrity of the livers. Livers receiving entrapped or free pepstatin were further perfused with a single pass of 0.9% NaCl (100 ml) at 130 min, and then homogenised in 5 volumes of 0.25 M sucrose 50 mM potassium phosphate buffer, pH 7.5. Inhibition of cathepsin D in the homogenates was estimated by assaying aliquots before and after dialysis against 50 volumes of phosphate buffer, pH 7.5, with one change. At pH above 6.4, there is little binding of pepstatin to cathepsin D (C. G. Knight, personal communication) and thus dialysis allows recovery of activity. The enzyme in homogenates of livers receiving liposomal pepstatin was approximately 80% inhibited, and that from livers receiving free pepstatin was approximately 30% inhibited. These values probably overestimate the degree of inhibition achieved in the perfusion since homogenisation will bring all uncomplexed inhibitor into contact with enzyme (as indicated by the inhibition of homogenate enzyme observed after treatment with free pepstatin, in the absence of inhibition of protein degradation). The percentage rates produced by treatments (a)–(c) do not differ significantly from 100%, whereas those following (d) are significantly different from those following (c) ($P < 0.05$).

Furthermore, pepstatin seems not to permeate macrophage plasma membranes (personal communication from A. J. Barrett and C. G. Knight) or other cellular membranes (with the possible exception of those of erythrocytes infected with malarial parasites¹⁴). Therefore it probably does not permeate lysosomal membranes, as might be expected for a pentapeptide¹. Because of this impermeability it was possible to direct the pepstatin specifically to lysosomes by entrapping it in multilamellar liposomes¹⁵ which are known to enter liver lysosomes rapidly after intravenous injection¹⁶. After endocytosis by Kupffer and parenchymal cells¹⁶, secondary heterolysosomes containing liposomes are formed, and by subsequent fusions, the liposomes also reach autophagic secondary lysosomes¹⁶. This method of administration further reduces the possibility of inhibition of unknown carboxyl proteinases outside the lysosome, by limiting access of the inhibitor to the cytoplasm.

It has been shown⁴ that livers of rats prelabelled with ¹⁴C-valine (given 2–4 h previously) lose labelled valine to the medium on subsequent perfusion. During perfusion in the presence of 15 mM cold valine (to prevent reincorporation) the linear rate of output of radioactivity is proportional to the rate of proteolysis in the period from 60 to 180 min after commencement. Radioactive secretory proteins have left the liver, and been taken up again to a negligible extent⁴ (my unpublished work). The method therefore measures the rate of degradation of intracellular proteins. I have determined this rate during

a 45-min period, and at 105 min from the start of perfusion, added liposomes containing pepstatin, or control liposomes, or free pepstatin, to the medium, and measured the subsequent rate. Figure 1 shows the results of a pair of such perfusions, and Table 1 summarises the results.

In perfusions to which control liposomes or free pepstatin were added at 105 min, there was no significant difference between the rates before and after the addition (Table 1). In contrast, there was a highly significant inhibition by liposomes containing pepstatin (Fig. 1, Table 1). The lack of inhibition by free pepstatin (Table 1b) supports the suggestion that pepstatin does not permeate liver cell membranes.

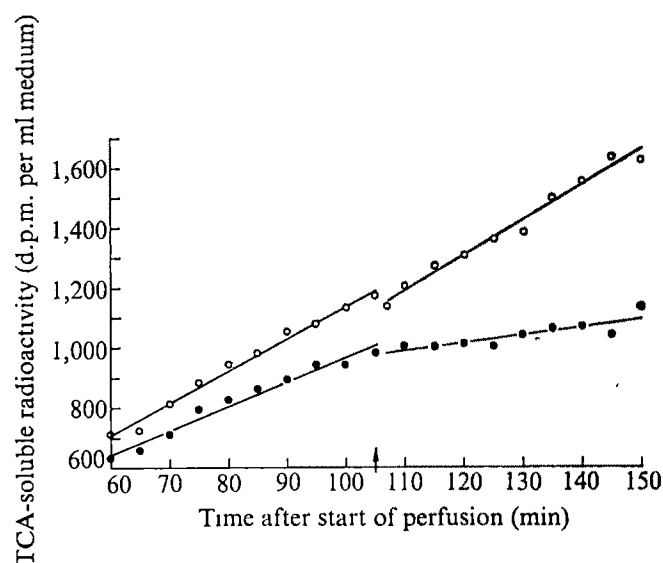


Fig. 1 Livers from rats prelabelled (2.5–3.5 h) with L-1-¹⁴C-valine (25 μ Ci) were perfused by a minor modification (addition of amino acids to a quarter of their concentration in DMEM, Flow Laboratories, Irvine, Scotland, and glucose, 14 mM, to the medium) of an established technique¹⁷. Medium radioactivity soluble in 5% trichloroacetic acid–10 mM valine (cold) was determined in samples (0.5 ml) taken every 5 min. The arrow indicates the addition of cationic liposomes (20 μ mol phosphatidyl choline, 20 μ mol cholesterol and 10 μ mol stearylamine, in 2 ml 5 mM potassium phosphate buffer, pH 7.0) which probably maintain a fairly constant intrahepatic concentration after a short time¹⁸, and which may reach parenchymal¹⁶ in addition to Kupffer cells. To prepare liposomes¹⁸, lipids (in chloroform-methanol, 2:1) were dried on to the surface of a glass homogeniser in a nitrogen stream, and desiccated under vacuum. After homogenisation (ten passes of a tight pestle) in buffer with pepstatin, 50 μ g ml⁻¹ (●; see also Table 1d) or in buffer alone (○; Table 1c), they were sonicated for 10 s, and used without further treatment. Entrapment of 20–30% of the pepstatin was demonstrated by gel filtration on Sepharose 4B in 0.9% NaCl, which separates free pepstatin from liposomes. Pepstatin was estimated¹⁹ by measuring the activity of various known amounts of human cathepsin D in the presence of column fractions treated with 0.1% Triton X-100 to lyse liposomes. Latency of liposomal pepstatin was confirmed by refiltering entrapped pepstatin after lysis. Because a similar degree of capture of the anionic pepstatin was also observed with anionic liposomes (10 μ mol phosphatidic acid in place of stearylamine) its association with liposomes was probably not merely electrostatic. Line fitting was done by the method of least squares.

These results constitute the first direct evidence that degradation of intracellular proteins in liver is to a large extent performed by lysosomes. They also re-emphasise the role of cathepsin D in digestion of complex substrates, complementing work on cathepsin B₁ and other proteinases⁵. Although perfusion itself increases intracellular proteolysis⁴, the inhibition by pepstatin is great enough to indicate inhibition of both basal and stimulated proteolysis.

It seems probable that entry of proteins into lysosomes is normally the rate limiting step in lysosomal protein breakdown^{1,6}, particularly in view of the lack of evidence for escape of proteins from lysosomes. In the presence of inhibitors of lysosomal

proteinases, intralysosomal proteolysis is retarded, either leading to an intralysosomal accumulation of protein or perhaps to a feedback inhibition of uptake. Either of these events may occur also when lysosomal proteinases are genetically defective; that this has not been detected in man¹⁹ implies they are lethal in early embryonic life. The mechanisms of uptake, and their control and specificity, now deserve considerable further attention⁶.

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Synthetic substrate for cyclic AMP-dependent protein kinase

Most hormones and neurotransmitters are thought to stimulate the synthesis of adenosine 3',5'-cyclic monophosphate (cyclic AMP) which in turn activates protein kinases¹. On activation, the protein kinases transfer the terminal phosphate group of ATP to serine or threonine residues in enzymic or membrane proteins involved in metabolic regulation, thereby either activating or inactivating them. Only a few key proteins are phosphorylated^{1,2} which raises the question of the molecular basis for the recognition of a particular serine by the cyclic AMP-dependent protein kinases. Cohen *et al.*² considered this problem with respect to the phosphorylation of phosphorylase kinase and glycogen synthetase and concluded that the cyclic AMP-dependent protein kinase recognises some specific three-dimensional configuration formed by a particular amino acid sequence at the site of phosphorylation. The features of this structure, however, remain to be defined. It has been shown³ that small peptides from myelin basic protein could act as substrates, thus reducing the number of parameters for consideration.

A major site of phosphorylation by cyclic AMP-dependent protein kinase in intact myelin⁴, isolated myelin basic protein⁵, and in peptic peptides⁶ was serine 110 in the sequence Gly–Arg–Gly–Leu–Ser–Leu. Here we examine the phosphorylation of a synthetic peptide equivalent to amino acid residues 106–113 in the basic protein of human myelin⁷. The peptide is thus the first synthetic substrate for the cyclic AMP-dependent protein kinases which seem to be so important in regulatory processes.

The peptide Gly–Arg–Gly–Leu–Ser–Leu–Ser–Arg was synthesised by the solid phase technique⁸, as modified for auto-

matic synthesis (M. D. Geier, F. S. Geier, and J.D.Y., unpublished). The double coupling programme included chloroform as solvent, dicyclohexylcarbodiimide as coupling agent, 25% trifluoroacetic acid to remove *t*-butyloxycarbonyl protecting groups and anhydrous HF at 0 °C for 30 min to cleave the peptide from the resin and to remove the O-benzyl and nitro groups from serine and arginine respectively. Initial purification was on Sephadex G-15 and the yield was 20%. The peptide was stained with ninhydrin and had a mobility of +0.59 relative to aspartic acid⁹ on high-voltage electrophoresis using Whatman 3MM paper in pyridine–acetate buffer, pH 6.5. Two minor contaminants with relative mobilities of +0.34 and +0.65 were removed by this technique. (These contaminants, which also acted as substrates for the protein kinase, presumably arose from the incomplete removal of the nitro-protecting group on the C-terminal arginine¹⁰.) The purified peptide on analysis contained equimolar ratios of its four constituent amino acids.

Protein kinases were prepared from bovine cardiac muscle and brain^{3,11} and further purified by gel filtration on Sepharose 4B in 5 mM potassium phosphate buffer, pH 7.0, containing 2 mM EDTA. The kinases were assayed with histone and with myelin basic protein as substrates and cyclic AMP produced a stimulation between four- and sevenfold.

In the experiments with synthetic peptide, the electrophoretic assay for protein kinase activity was used³. The actual incubation conditions are described in Fig. 1 and below. The peptide (250 nmol) was incubated with γ -³²P-ATP (1 μ mol, 8.7×10^6 c.p.m.), cardiac protein kinase (8.5 μ g), 4 nmol cyclic AMP, 1.4 μ mol magnesium acetate, 120 nmol EGTA, in 30 mM sodium acetate buffer, pH 6.5, in a total volume of 350 μ l at 30 °C for 2 h. The mixture was fractionated by preparative paper electrophoresis as described above and the paper sheet

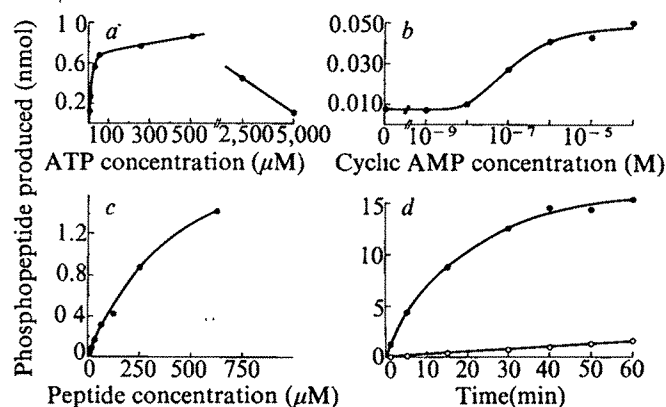


Fig. 1 Production of phosphorylated peptide Gly–Arg–Gly–Leu–Ser(P)–Leu–Ser–Arg; ordinate refers to the total nmol of phosphopeptide produced at 30 °C in each incubation tube in 5 min or, in *d*, for the time indicated. In *a*, *b* and *c* 10 μ l of each of the solutions (i), (ii) and (iii) were incubated in separate tubes with 10 μ l of cardiac protein kinase (85 μ g ml⁻¹). Incorporation of ³²P into the peptide was assayed on 25 μ l samples by the electrophoretic method³. *a*, Effect of ATP: (i) peptide in water (10 nmol per 10 μ l); (ii) 14 mM magnesium acetate, 1.2 mM EGTA and 40 μ M cyclic AMP, adjusted to pH 6.5, and (iii) γ -³²P-ATP (3.0×10^6 c.p.m. per 10 μ l) mixed with ATP to give the final concentration indicated in 100 mM sodium acetate buffer, pH 6.5. *b*, Effect of cyclic AMP: (i) peptide in water (3.1 nmol per 10 μ l); (ii) as in *a* but incorporating cyclic AMP to give the final concentration indicated; and (iii) 277 μ M γ -³²P-ATP (2.5×10^6 c.p.m. per 10 μ l) in buffer as in *a*. *c*, Effect of peptide: (i) peptide in water to give the final concentration indicated; (ii) as in *a*; and (iii) 2 mM γ -³²P-ATP (3.0×10^6 c.p.m. per 10 μ l) in buffer as in *a*. *d*, Effect of time and cyclic AMP. Two tubes were prepared with the following solutions (i) 5 μ l peptide in water (50 nmol per 10 μ l), (ii) 50 μ l as in *a* or in the second tube as in *a* but without cyclic AMP present; (iii) 50 μ l 2 mM γ -³²P-ATP (1.4×10^6 c.p.m. 50 μ l) in buffer as in *a*, and (iv) 50 μ l cardiac protein kinase. At times indicated 20 μ l were removed for assay.

Table 1 Sites of phosphorylation by cyclic AMP-dependant protein kinases

Substrate		Sequence	Sources of kinase	Ref.
Non-globular substrates				
Histone F1		Arg-Arg-Lys-Ala-Ser-Gly-Pro *	Liver	22, 23†
Histone F2b		Ser-Arg-Lys-Glu-Ser-Tyr-Ser *	Lymphocytes	24
Myelin basic protein	Site 1	Gly-Arg-Gly-Leu-Ser-Leu-Ser *	Muscle	
	Site 2	Gln-Arg-His-Gly-Ser-Lys-Tyr *	and	
	Site 3	Arg-His-Arg-Asp-Thr-Gly-Ile *	Brain	4, 5, 6
RCMM Lysozyme†	Site 1	Tyr-Arg-Gly-Tyr-Ser-Leu-Gly *		
	Site 2	Arg-Asn-Thr-Asp-Gly-Ser-Thr-Asp *	Muscle	21
Protamine	Site 1	Arg-Arg-Arg-Ser-Ser-Ser-Arg * * *		
	Site 2	Ala-Arg-Arg-Val-Ser-Arg-Arg *	Testis	25, 26
Globular substrates				
Phosphorylase kinase	α subunit	Arg-Leu-Ser-Ile-Ser Lys		
	β subunit	or-Gln-Ser-Gly-Ser- or-Tyr Arg Ile	Muscle	2
Troponin I	Site 1	Val-Arg-Met-Ser-Ala-Asp *		
	Site 2	Ser-Val-Met *	Muscle	18, 19
Pyruvate kinase (Liver)		Leu-Arg-Arg-Ala-Ser-Leu *	Liver	17
Glycogen synthetase		Lys or-Gln-Ile-Ser-Val Arg	Muscle	27

*Amino acid residue phosphorylated.

†RCMM Lysozyme, reduced carboxymethylated maleylated lysozyme.

†S. C. Rall and R. D. Cole, in ref. 23.

subjected to autoradiography. A zone of radioactivity with a relative mobility of +0.19 was detected. A sample of this zone stained the typical yellow colour given by glycyl peptides. The zone was eluted with 0.01 M NH_4OH and from the incorporated radioactivity and amino acid analysis 1 mol of phosphate per mol of peptide was calculated; the total yield of phosphopeptide was 71 nmol. Successive Edman degradations of the phosphopeptide and electrophoresis of the products at pH 6.5 showed that the phosphate was present as shown in the sequence Gly-Arg-Gly-Leu-Ser(P)-Leu-Ser-Arg. This result was supported by studies with aminopeptidase and thermolysin. An identical labelling pattern was obtained with bovine brain protein kinase.

In these experiments Offord's formula⁹ was used to follow the change in electrophoretic mobility on the addition or removal of charged groups. We found that the average value for the change in charge on the addition of a phosphate group to a peptide was -1.35 at pH 6.5 in pyridine acetate buffer. When the phosphoserine was N-terminal, however, the phosphate group carried a more negative charge (approximately -1.6).

In kinetic experiments the cardiac kinase was used. Human myelin basic protein was used at the same molar concentration as the peptide to enable a comparison to be made. The effect of increasing ATP concentration on the production of phosphopeptide is shown in Fig. 1*a*. Michaelis-Menten kinetics were exhibited and from a double reciprocal plot a K_m value of 2.8×10^{-5} M was determined for ATP, similar to values reported for various protein kinases with proteins as substrates^{12,13}. The K_m found with myelin basic protein was 6.8×10^{-6} M. At high concentrations of ATP, in excess of Mg^{2+} , there was a marked inhibition of the production of

phosphopeptide. A similar effect observed with phosphorylase kinase^{14,15} was attributed to the complexing of Mg^{2+} by ATP.

Half-maximal stimulation by cyclic AMP was produced at 10^{-7} M for both the synthetic peptide (Fig. 1b) and myelin basic protein as substrate. Values ranging from 0.4×10^{-7} to 3×10^{-7} M have been reported for a bovine cardiac protein kinase with histone (calf thymus type IIA) as substrate^{12,13}.

The effect of increasing concentration of synthetic peptide is shown in Fig. 1c. Again Michaelis-Menten kinetics were exhibited and from the linear double reciprocal plot a K_m of 2.4×10^{-4} M for the synthetic peptide with the cardiac enzyme was found. This value is similar to that reported for a peptic peptide substrate³. With myelin basic protein as substrate the K_m was lower (5.3×10^{-5} M). Miyamoto and Kakiuchi¹⁶ found a K_m of 2×10^{-5} M for the protein with a brain kinase. The V_{max} for the synthetic peptide was 1.47 nmol ³²P incorporated per 5 min and for the protein 0.36 nmol ³²P incorporated per 5 min. It should be emphasised that in the protein both threonine and serine sites are subject to phosphorylation⁵ (Table 1), whereas with the synthetic peptide only a single site was phosphorylated, thus the comparison of K_m and V_{max} is of limited value.

The effect of increasing time of incubation on the production of phosphopeptide in the absence and presence of cyclic AMP is shown in Fig. 1*d*. In 1 h very little phosphorylation of the peptide was observed in the absence of cyclic AMP. Thus it seems that the peptide is not able to dissociate the catalytic and regulatory subunits. A similar result was obtained with intact myelin basic protein whereas other studies¹⁶ have reported activation of protein kinase by both this protein and histone. But another sample of the same cardiac kinase as used above that had been frozen and thawed repeatedly showed less cyclic

AMP dependence and much greater activation with time when incubated without cyclic AMP (compare ref. 12).

The synthetic peptide was digested with proteolytic enzymes⁷ or subjected to Edman degradation⁷ and the products examined for substrate activity. Removal of the C-terminal arginine with carboxypeptidase B yielded a peptide, with relative mobility +0.38, which was readily converted to a phosphopeptide with relative mobility -0.06. From the N-terminal end glycine could be removed by Edman degradation without apparent effect on substrate activity, whereas the arginine was essential for activity. This requirement for arginine was confirmed by tryptic digestion, which also destroyed the capacity of the peptide to be phosphorylated. It could be argued that the loss of activity was simply due to the resultant shortening of the peptide but the need for arginine, rather than a neutral amino acid, is supported by the following observations. Although there are numerous serine and threonine residues within quite large tryptic peptides from myelin basic protein, only a few peptides in the tryptic digest were phosphorylated. All the peptides which were not phosphorylated had lysine or arginine to the C-terminal side of the serines whereas those that were phosphorylated contained arginine to the N-terminal side in trypsin resistant bonds⁶. One of these was the peptide Gly-N^G-monomethyl Arg-Gly-Leu-Ser-Leu-Ser-Arg. Preliminary evidence indicated that when the arginine was in the N^G,N^G-dimethyl form phosphorylation of the serine was prevented⁵. Moreover an examination of amino acid sequences around the site phosphorylated by cyclic AMP-dependent protein kinases in a number of non-globular and globular proteins (Table 1) shows that arginine is frequently found close to the serine that is phosphorylated.

Table 1 summarises the amino acid sequences in proteins phosphorylated by cyclic AMP-dependent protein kinases from a variety of tissues. Although in several cases the sequence Arg-X-Y-Ser-Z can be identified, there are also some instances where the arginine is only one residue removed from the serine or one further away. Hjelmquist *et al.*¹⁷ suggested a similar pattern and also that the serine should be surrounded by hydrophobic residues. The latter concept is not fully supported by the data in Table 1. It is puzzling that phosphorylase kinase phosphorylates the same type of site in phosphorylase b and in troponin I yet these sites are not those phosphorylated by the cyclic AMP-dependent protein kinases^{18,19}, which act on other serines in these proteins (Table 1).

Using probability factors listed by Chou and Fasman²⁰ for each amino acid at each position, 1-4, in a β bend, the probability of the sequences listed in Table 1 taking up a β -bend conformation was calculated. Although in several, a high probability emerged for the serine being in position 4 in a β bend, such high probability values were also obtained for sequences around serines in the basic protein which are known not to be phosphorylated.

Sites of phosphorylation in myelin basic protein were independent of the source of the kinase; purified kinases from rabbit skeletal muscle and bovine brain and cardiac muscle all produced the same labelling pattern (Table 1). In some substrates more than one site is phosphorylated at variable rates^{2,4,5,21}; however, no feature is apparent in the more readily phosphorylated sequences. This would suggest that once some minimum requirement is met, the rate of phosphorylation is governed by other factors.

Substrates in Table 1 have been categorised as non-globular and globular but no consistent differences are apparent in the sequences around the site of phosphorylation. Bylund and Krebs²¹ have shown that lysozyme, phosphorylase b and bovine serum albumin contain certain serine residues which are quite inactive as substrates in the native form, but which are readily phosphorylated when the proteins are denatured. This implies that in the native structure the sequence around these particular serines is constrained from taking up the conformation necessary for interaction with the active site of the enzyme. The sequence Arg-X-X-Ser is not a rare sequence

in proteins which do not act as substrates in their native form². Whether the failure of the kinase to phosphorylate these serines is due to the lack of accessibility or to the arginine being, too remote spatially from the serine remains to be determined. Whatever the three-dimensional arrangement of the enzyme-substrate complex, however, we predict the need for a basic amino acid in close proximity to the serine to be phosphorylated. Chemical and physical studies with other synthetic peptides could help solve this problem.

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Tubulin requires an accessory protein for self assembly into microtubules

BORISY and Taylor have demonstrated a correlation between the presence of the colchicine-binding protein, tubulin, and the microtubular system of eukaryotic cells¹. Tubulin assembles *in vitro* into microtubules when a brain homogenate is warmed to 37 °C and is supplied with a cofactor, GTP (refs 2 and 3). These microtubules dissociate into tubulin subunits when the solution is chilled to 0 °C (refs 3 and 4), or Ca²⁺ is introduced^{2,4}. Tubulin can thus be purified by repeated cycles of assembly and disassembly^{4,5}. We have found, and others report similarly⁶⁻¹⁰, that some very high molecular weight polypeptides copurify with the tubulin in these conditions.

We have examined the role of these polypeptides in the assembly process and found that separation of the high molecular weight fraction from tubulin prevents self assembly of the tubulin; the colchicine-binding property of the tubulin is un-

affected. The high molecular weight fraction restores assembly competence when added back to the tubulin.

A preparation (for details, see Fig. 1) containing tubulin and the high molecular weight proteins was competent to reassemble into microtubules. We found two characteristic forms present in electron micrographs: 50-nm rings and 10-nm flexible fibres.

Tubulin has previously been reported to exist as dimers (molecular weight 110,000, $s_{20,w}6S$) and in an aggregate form of defined structure, a 49-nm ring complex ($s_{20,w}36S$)^{12,13}. Only 36S tubulin, but not 6S can assemble into microtubules^{12,13}. The 36S form is dissociated by high salt concentration (> 0.8 M NaCl) and reforms if the salt is dialysed away.

We have used this property to separate the constituents of the 36S particle. First, the microtubule pellet was dispersed in homogenising buffer containing 1 M NaCl at 0 °C. The treatment dissociated the 50-nm rings but not the 10-nm fibres, which were removed by centrifugation at 220,000g and 0 °C for 2 h. The supernatant, which was competent to assemble microtubules after the salt was removed, contained tubulin and only one of the high molecular weight polypeptides (Fig. 1d). The proteins were resolved by gel filtration, in the presence of 1 M NaCl on Sepharose 4B (Fig. 2a) or Sephadex G200 (similar, but not shown).

To remove NaCl the fractions were dialysed against medium containing 30 mM MES buffer; 0.2 mM $MgCl_2$; 1 mM EGTA;

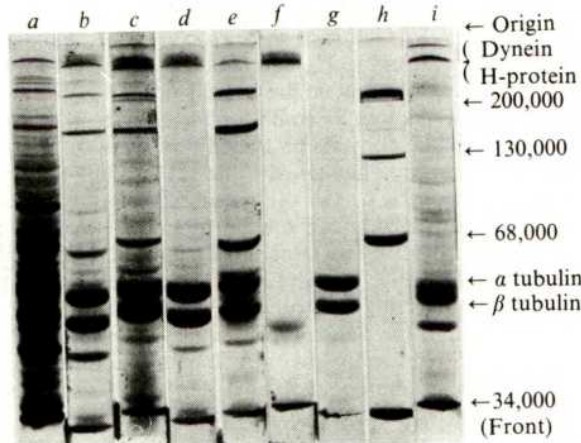


Fig. 1 Analysis of microtubule proteins by SDS-polyacrylamide gel electrophoresis. *a*, Whole brain supernatant; *b*, pellet after first assembly step; *c*, supernatant after three cycles of assembly-disassembly, without NaCl; *d*, supernatant after two cycles, 1 M NaCl in second disassembly; *e*, pellet from second cycle disassembly, 1 M NaCl; *f*, peak H from Sepharose 4B column; *g*, peak T from Sepharose 4B column; *h*, marker proteins: 34,000 aspartate transcarbamylase C subunit; 68,000 bovine serum albumin; 130,000 β -galactosidase; 200,000 myosin; *i*, flagellar proteins from *Naegleria gruberi*. Gel electrophoresis was carried out using the procedure of Laemmli¹¹, except that running gels contained only 6.7% acrylamide. Tubulin was prepared from rabbit brains following a method adapted from Shelanski⁹. Excised brains (about 9 g each) from adult rabbits were homogenised at 0 °C in a medium (12 ml per brain) containing 40 mM 2-(N-morpholino) ethanesulphonic acid, adjusted to pH 6.6 with NaOH (MES buffer); 0.3 mM $MgCl_2$; 1.5 mM ethyleneglycol-bis-(2-aminoethyl ether)N,N'-tetra-acetic acid (EGTA); 5 mM 2-mercaptoethanol and 1 mM GTP. The homogenate was centrifuged at 48,000g and 0 °C for 60 min. Glycerol was added (1 ml per 3 ml supernatant) and GTP was replenished (1 μ mol per ml supernatant) and the mixture was incubated at 37 °C for 30 min. Microtubules were collected by centrifugation at 100,000g and 25 °C for 60 min. The pellet was resuspended in homogenising buffer (1.5 ml/brain) at 0 °C to disassemble the microtubules, and was dispersed by agitation and expulsion through a syringe. After 30 min at 0 °C, the suspension was centrifuged at 120,000g and 0 °C for 40 min. Glycerol and GTP were added to the supernatant and microtubules were assembled by incubation at 37 °C as above. The second-stage microtubule pellet was resuspended in dissociation buffer (1 ml/brain) containing 40 mM MES buffer; 0.3 mM $MgCl_2$; 1.5 mM EGTA; 1.5 M NaCl; 5 mM 2-mercaptoethanol and 1 mM GTP. After 1 h at 0 °C, glycerol was added to a final concentration of 20% (v/v).

5 mM 2-mercaptoethanol and 4 M glycerol. GTP was added to the retentates to a concentration of 1 mM and the preparations were examined for microtubule assembly by electron microscopy (Fig. 3). Tested individually, peak H (from Fig. 2a), which contained the high molecular weight protein, and peak T, which contained tubulin, failed to form ring complexes at 0 °C and

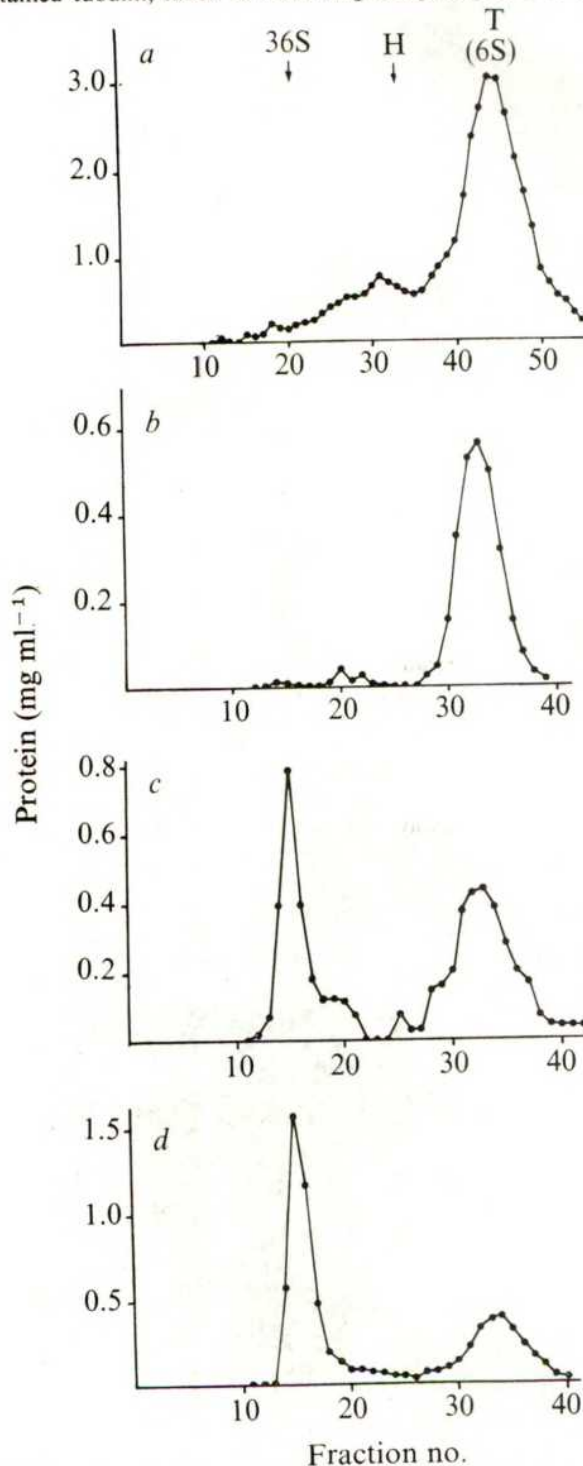


Fig. 2 Fractionation by gel filtration of microtubule disassembly products. *a*, Microtubules were disassembled at 0 °C, and products were dissociated by 1 M NaCl. Components were resolved by gel filtration in Sepharose 4B equilibrated in buffer containing 30 mM MES, pH 6.6; 1 mM EGTA; 0.2 mM $MgCl_2$; 1 M NaCl; 5 mM 2-mercaptoethanol and 20% (v/v) glycerol. Peak H contains high molecular weight protein and peak T corresponds to 6S tubulin; *b*, rechromatography of peak T after removal of NaCl, by gel filtration in Sepharose 4B, buffer as for *a*, but without NaCl; *c*, rechromatography of mixture of 1 mg H + 5 mg T, preincubated with 1 mM GTP at 0 °C, column conditions as for *b*; *d*, microtubules disassembled at 0 °C, and products fractionated by gel filtration on Sepharose 4B without NaCl dissociation of 36S rings, buffer as for *b* and *c*.

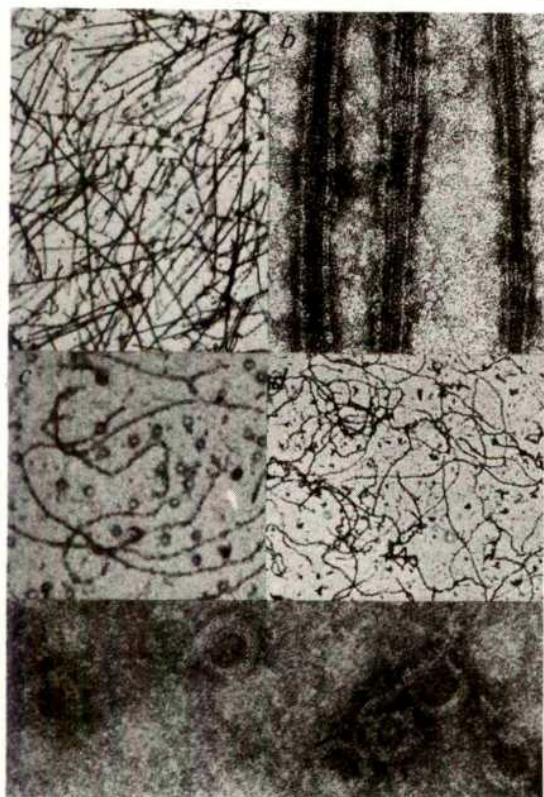
microtubules after incubation at 37 °C. Tubulin alone was passed again through Sepharose 4B, this time in the absence of NaCl, and eluted in the normal position for 6S dimer (Fig. 2b), indicating that 36S ring complexes had not formed. A mixture of tubulin and high molecular weight protein, allowed to reassociate in the cold in the absence of salt, formed the aggregate which eluted from Sepharose 4B in the position corresponding to 36S rings (Fig. 2c and d). Rings were observed in electron micrographs of the 36S peak (Fig. 3).

Mixtures of the two components incubated at 37 °C formed microtubules in large numbers ($> 10^3/0.003 \text{ mm}^2$ grid). In some cases, the 6S tubulin fraction prepared by Sepharose 4B chromatography formed limited numbers of microtubules ($< 30/0.003 \text{ mm}^2$ grid), but the high molecular weight protein was detected as a contaminant in these samples. Sephadex G200 generally gave a superior resolution of the components.

Early attempts to reconstitute an assembly-competent tubulin-accessory protein complex were unsuccessful until we included 2-mercaptoethanol in the buffers.

We estimate the molecular weight of the accessory protein to be about 360,000 from its mobility in SDS-polyacrylamide gel electrophoresis, by extrapolation from myosin (200,000), our largest available marker. The band matches the mobility of dynein isolated in flagellae from *Naegleria gruberi*. It seems likely that our microtubule accessory protein corresponds to the dynein-like proteins identified in brain tissue, which were also found associated with tubulin purified by the assembly-disassembly cycling procedure^{8,9}. These authors failed to find significant ATPase activity, characteristic of flagellar dynein, associated with their brain "dynein".

Fig. 3 Electron micrographs of tubulin preparations: a, mixture containing tubulin (2 mg ml⁻¹) and peak H protein (0.4 mg ml⁻¹) incubated for 30 min at 37 °C with 1 mM GTP ($\times 9,720$); b, same preparation at higher magnification, showing subunit structure of microtubules ($\times 184,200$); c, supernatant after microtubule disassembly in the third cycle of purification (see Fig. 1c for protein composition): fibres lack any subunit structure similar to microtubules ($\times 40,090$); d, microtubule disassembly in 1 M NaCl, rings have disappeared but fibres remain ($\times 20,350$); e, supernatant after 220,000g centrifugation of NaCl-dissociated tubulin, dialysed and incubated at 0 °C with 1 mM GTP (protein composition shown in Fig. 1d) ($\times 214,800$). Preparations were negatively stained with 0.7% uranyl acetate, on carbon-coated Parlodion grids, with 25 $\mu\text{g ml}^{-1}$ bacitracin as spreading agent.



The extreme size of the polypeptide causes difficulties in gel electrophoresis in many conventional systems and the protein penetrates poorly in gels of too high acrylamide concentration ($> 7\%$) or of too high bisacrylamide-acrylamide ratio. Either condition prevents formation of a distinct band in electrophoresis gels and would account for the failure of other workers to detect the accessory protein¹³.

We have evidence that the accessory protein is incorporated into the structure of microtubules rather than acting as an initiating factor or assembly catalyst. *In vitro* assembled microtubules band in sucrose density gradients at 1.289 g cm⁻³ (R.A.B.K. and R.H.H., unpublished) and both tubulin and the high molecular weight protein coincide with the band. Similarly, the accessory protein coelutes with the ring complex from a column of Sepharose 4B (Fig. 2d), although there is considerable difference in molecular size. When limiting amounts of accessory protein are added to pure tubulin, there is limited assembly into microtubules. These data are, however, based on electron microscopy which is only semi-quantitative and it has not been possible to determine an optimum stoichiometry for maximum reassembly of microtubules. The protein comprises about 15% by weight of intact microtubules (data derived from Fig. 2a), which corresponds to one 360,000 molecular weight protein per forty 55,000 molecular weight tubulin subunits, a ratio which suggests that each ring complex¹³ contains one accessory protein.

The results we describe here confirm the report by Weingarten *et al.* that a protein factor is required in addition to tubulin for microtubule assembly¹⁴. Weingarten found the assembly-promoting activity, tau factor, in a heterogeneous fraction containing many proteins which were not further resolved. Our high molecular weight protein has similar properties to the tau factor in that it permits assembly of 6S tubulin into microtubules at 37 °C and converts 6S to 36S tubulin at 0 °C.

Electron microscopy has failed to detect any large globular protein corresponding to the estimated size of the accessory microtubule protein, although tubulin monomers (55,000) can be distinguished in the structure of the microtubule. We speculate therefore that the accessory protein exists as a thin filament interconnecting the subunits, both in the complete microtubule and in the ring complex. Burns and Pollard⁸ have calculated the Stokes radius of their dynein-like protein from brain microtubules and conclude that it is highly asymmetric. One interesting possibility that emerges is the potential for different forms of accessory protein to modulate the properties of the resulting microtubule, thereby enabling identical tubulin subunits to form functionally distinct classes of microtubule. In this context, the amino acid sequence of tubulin has been found to be highly conserved in examples from widely divergent taxa¹⁵.

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'Single-stranded' DNA from ϕ X174 and M13 is cleaved by certain restriction endonucleases

DURING a systematic survey of the substrate specificities of a variety of restriction endonucleases, it was discovered that the *Haemophilus aegyptius* restriction endonuclease III (*Hae*III) specifically cleaved 'single-stranded' DNA from ϕ X174 and M13. Since these DNAs are not double-stranded¹ and restriction enzymes recognise duplex DNA with twofold sequence symmetry², this result was unexpected. This finding is significant with regard to the conformation of single-stranded viral DNA as well as the biochemical mechanism and physiological role of restriction enzymes.

Figure 1 shows a polyacrylamide gel electrophoretogram of the fragments produced by *Hae*III on ϕ X174 replicative form (RF), ϕ X174 (+) strand and M13 (+) strand DNA. For the duplex ϕ X174 RF DNA, nine bands are visible; the smallest fragment is not visible and the sixth band is a doublet thus totalling eleven fragments, as reported previously^{3,4}. Digestion of the 'single-stranded' ϕ X174 (+) DNA with *Hae*III produced eleven visible bands and similar digestion of M13 (+) DNA produced nine (Fig. 1). It was shown previously⁵ that M13 RF was cleaved by *Hae*III into ten fragments. The rate of cleavage of the 'single-stranded' DNAs was not as great as for the duplex RF; a 16 h digestion of the (+) strand DNAs was necessary to give a limit digest, whereas a 1 h period sufficed for the duplex RF. The reason for this difference in rates is unknown at present. Similar patterns were obtained when the *Streptococcus faecalis* restriction endonuclease I (gift of R. Wu) replaced *Hae*III. The cleavage site for both enzymes is GGCC.

Regions of duplex helical structure in the (+) strand DNAs (refs 6-8) seem to provide suitable sites for the specific cleavages. Heat-treated λ plac 5 DNA was not degraded, whereas the native double-stranded DNA was degraded to more than 50 specific fragments by *Hae*III in identical conditions. If, however, ϕ X174 (+) DNA was heated (98 °C for 5 min in 5 mM sodium phosphate (pH 7.4)-10 μ M EDTA) followed by quick chilling in ice before treatment with *Hae*III, it was degraded similarly to the untreated DNA. The ϕ X174 DNA seemed to reform appropriate duplex restriction sites readily whereas λ DNA did not. We cannot at present rule out the possibility that a single-stranded non-helical DNA is cut at a slow rate; however, the necessity of a duplex helical structure was shown also by studies with *Hpa*II (B. Baumstark, R. Roberts and U. Rajbhandary, unpublished) and *Eco*RI (ref. 9) on appropriate synthetic substrates. Details of the mechanism of cleavage of 'single-stranded' DNAs are uncertain at present and are currently being investigated.

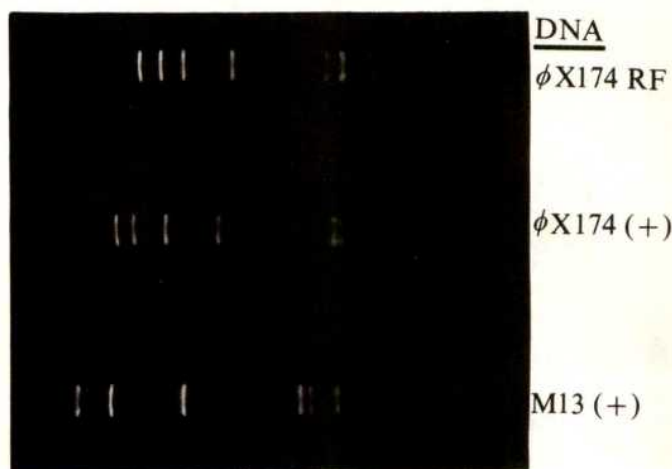


Fig. 1 Polyacrylamide gel electrophoresis of ϕ X174 RF, ϕ X174(+), and M13 (+) DNAs digested with *Hae*III. Both ϕ X174 DNAs contained *am*3 and *ts*41D mutations (gifts of J. Dodgson and I. Nes); M13 (+) DNA was prepared as described previously¹⁰. The purification of *Hae*III was essentially as described by Roberts *et al.*¹¹ but in addition, the final enzyme stock solution was made 0.5 mg ml⁻¹ in bovine serum albumin. Each DNA sample (2 μ g) was incubated at 37 °C for 18 h in a 50 μ l reaction mixture containing 6 mM Tris-HCl (pH 7.9), 6 mM MgCl₂, 1 mM dithiothreitol and 6 U (ref. 11) of *Hae*III. Digestion was terminated by addition of EDTA to 50 mM. Each sample was made 15% (v/v) in glycerol and 0.03% (w/v) in bromophenol blue, then subjected to electrophoresis in 4% polyacrylamide cylindrical gels in Tris-borate EDTA buffer (90 mM Tris-borate (pH 8.3), 2.5 mM EDTA)¹² for 4 h at 200 V. The polyacrylamide gels (0.6 \times 10 cm) were 3.8% (w/v) in acrylamide, 0.2% (w/v) in N,N'-methylene bisacrylamide (Bis), and were prepared in Tris-borate EDTA buffer-25% glycerol. After electrophoresis the gels were stained for 10 min with 10 μ g ml⁻¹ ethidium bromide and photographed during ultraviolet irradiation. Electrophoresis was from left to right; bromophenol blue migrated to 90% of the length of the gel.

*Hae*III specifically cuts the 'single-stranded' DNA into fragments of the same size as produced by cleavage of the RF. Figure 2 shows a formamide-polyacrylamide gel electrophoretogram of parallel *Hae*III digests of ϕ X174 duplex and 'single-stranded' DNA. The patterns on these denaturing gels are superposable except for the presence of two additional fragments (450 and 150 nucleotides in length; see arrows, Fig. 2) in the (+) strand pattern. It is thus assumed that both the RF and (+) strand are cleaved at the same GGCC sites, with the exception of the two additional fragments; the origin of these two fragments is unknown. The presence of the 450 and 150 nucleotide fragments was also observed as the fifth and eighth bands from the origin, respectively, in the non-denaturing gel (Fig. 1).

The following observations are consistent with the notion that *Hae*III, and not a contaminating enzyme, is specifically cutting the 'single-stranded' DNAs: 1, the similarity of fragment patterns for RF and (+) strand DNA (described here); 2, the

Table 1 Effect of restriction endonucleases on ϕ X174 and M13 DNAs

Enzyme	Recognition sequence	Number of fragments			
		ϕ X174 RF	ϕ X174 (+)	M13 RF	M13 (+)
<i>Haemophilus aegyptius</i> (<i>Hae</i>) III	GGCC	11	11	10	9
<i>H. haemolyticus</i> (<i>Hha</i>) I	GCGC	≥ 14	≥ 14	≥ 12	≥ 9
<i>Streptococcus faecalis</i> (<i>Sfa</i>) I	GGCC	11	+	10	+
<i>H. influenzae</i> R _d (<i>Hind</i>) II	GTPyPuAC	13	0	1	0
<i>H. parainfluenzae</i> (<i>Hpa</i>) II	CCGG	8	0	13	0
<i>Arthrobacter luteus</i> (<i>Alu</i>) I	AGCT	> 10	0	> 10	0
<i>H. aegyptius</i> (<i>Hae</i>) II	Unknown	≥ 7	0	≥ 6	0
<i>H. influenzae</i> R _d (<i>Hind</i>) III	AAGCTT	0	0	0	0
<i>Escherichia coli</i> R (<i>Eco</i> R) I	GAATTC	0	0	0	0

The enzyme reaction conditions used were those previously reported for each nuclease using duplex DNA as substrate. The number of fragments produced by nuclease digestion of the RF DNAs was taken from refs 3-5 and our results. A + indicates that a number of specific fragments were observed, but the determination of their number was complicated by the presence of exonuclease in the *Sfa*I. The number of fragments generated by *Hpa*II on M13 RF is assumed to be the same as described⁵ for *Hap*II.

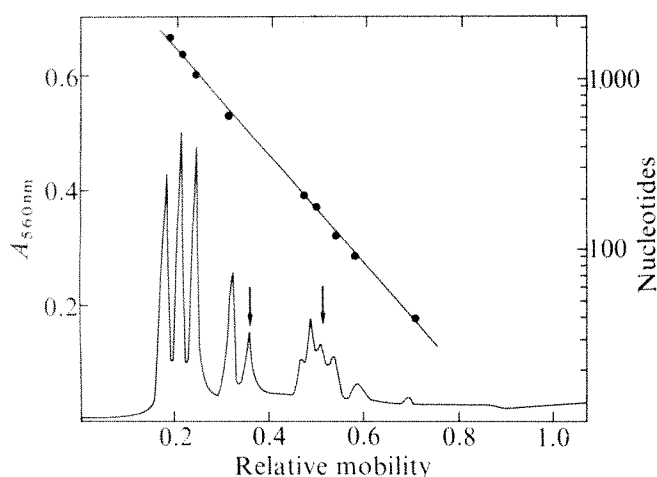


Fig. 2 Densitometric scan of *Hae*III fragments of ϕ X174 (+) DNA separated by formamide-polyacrylamide gel electrophoresis. ϕ X174 (+) DNA (3 μ g) was digested with *Hae*III as described in Fig. 1. Sodium acetate was added to the completed digest to 0.3 M, then 2.5 volumes cold ethanol was added. The DNA precipitate obtained after centrifugation was prepared for formamide-polyacrylamide gel electrophoresis as described by Maniatis *et al.*¹³. The DNA was dissolved in 25 μ l deionised formamide, heated at 100 °C for 2 min, then chilled in ice. Following the addition of 5 μ l of 25 mM sodium phosphate (pH 7.5), 75% glycerol and 0.2% bromophenol blue, the DNA sample was layered on a formamide-polyacrylamide cylindrical gel. The polyacrylamide gel (0.6 \times 10 cm) was 4.25% in acrylamide, 0.75% in Bis, and was prepared in 98% deionised formamide with 20 mM sodium phosphate (pH 7.5). Electrophoresis was in 20 mM sodium phosphate (pH 7.5) for 1.75 h at 200 V. The gel was then stained overnight with 0.005% Stain's All in 50% aqueous formamide as described¹⁴. After destaining, the gel was scanned at 560 nm in a Gilford spectrophotometer equipped with a linear transport (—). In a parallel experiment, 2 μ g of ϕ X174 RF DNA was digested with *Hae*III and subjected to formamide-polyacrylamide gel electrophoresis. The peaks of stained RF DNA fragments from a densitometric scan were plotted against the logarithm of the length of the fragments in nucleotides: Z1 (1690), Z2 (1350), Z3 (1025), Z4 (600), Z5 (215), Z6 doublet (175), Z7 (120), Z8 (90), and Z9 (40) (●). The fragment lengths were taken from Middleton *et al.*³. Abscissa is the distance of the DNA fragments from the origin, relative to bromophenol blue. Arrows indicate the *Hae*III fragments of ϕ X174 (+) DNA which do not coelectrophorese with *Hae*III fragments of ϕ X174 RF DNA; no undigested DNA was observed at the origins.

enzyme activity that specifically cleaves RF and (+) strand copurifies throughout the enzyme isolation; 3, three separate *Hae*III preparations in this laboratory cleave both RF and (+) strand DNAs as well as one provided by C. Hutchison, University of North Carolina; 4, the ability to cleave both RF and (+) strand DNAs was lost simultaneously on either dilution or storage at -20°C ; and, 5, *Sfa* I (GGCC) gives products which are virtually identical.

The ability of nine restriction endonucleases to cleave ϕ X174 and M13 DNAs is summarised in Table 1. In addition to *Hae*III and *Sfa*I, *Hha*I (gift of W. S. Reznikoff) also cleaves the (+) strand and RF DNAs. It is not, however, a general capacity of restriction endonucleases to cleave 'single-stranded' DNA since *Hind*III, *Hpa*II, *Alu*I (gifts of I. Nes, M. Mann and H. O. Smith, and L. Maquat and W. S. Reznikoff, respectively) and *Hae*II cut RF DNAs but not the (+) strands. The probability of the 50% G and 50% C containing recognition sites of *Hae*III (GGCC), *Sfa*I (GGCC), *Hha*I (GCGC) and *Hpa*II (CCGG) appearing in any duplex region of the (+) strand should be equal. The failure of *Hpa*II to cleave (+) strand indicates some additional recognition site requirement.

*Hind*III and *Eco*RI (gifts of I. Nes and B. Weissblum, respectively) cut neither the RF nor (+) strand DNAs. Therefore, no new sites for these enzymes are present in the 'single-stranded' DNA which were absent in the RF.

These studies demonstrate that *Hae*III, *Sfa*I and *Hha*I specifically cleave (+) strand DNA apparently because of the

presence of a substantial amount of helical structure in the 'single-stranded' DNA. That ϕ X174 and M13 (+) DNAs are not devoid of ordered structure was indicated in previous studies⁶⁻⁸. Many types of folded structures are possible and all would provide identical fragments for any given enzyme, providing that all potential cleavage sites are in helical regions for each type. DNA structural considerations are not, however, the sole determinant of (+) strand cleavages; enzyme specificity also must be important since *Hae*III, *Sfa*I and *Hha*I digest (+) DNA whereas *Hpa*II gives no cleavages.

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DNA breakage caused by dimethyl mercury and its repair in a slime mould, *Physarum polycephalum*

A PRELIMINARY report from our laboratory showed that dimethyl mercury produced a radiomimetic breakage of slime mould DNA and suggested that the damage was repaired enzymatically¹. This communication further characterises the DNA damage and establishes that breakage is independent of DNA replication. It provides conclusive evidence for the existence of a dimethyl mercury repair system and shows that strains of *Physarum* differing in geographical origin have widely different sensitivity to dimethyl mercury damage.

The first set of experiments determined the S value and thus the molecular weight of *Physarum* DNA. Brewer's sucrose gradient techniques² were used, with nuclear isolation media of three different pH values. The observed S value of single-stranded DNA increased with increasing pH in the nuclear isolation medium. At pH 7.5, the calculated molecular weight of single-stranded DNA was comparable with Brewer's value² of 6.3×10^7 . At pH 8.0, 3.77×10^8 was obtained for single-stranded DNA, while at pH 9.0 it was 6.3×10^9 . S values were obtained using the modified Burgi, Hershey relationship³ and molecular weight calculations were based on Studier's⁴ relationship for DNA in alkaline sucrose solutions. As the molecular weight of DNA in each slime mould nucleus is 6.0×10^{11} (ref. 5), and the accepted chromosome number is 50 (ref. 6), the average weight of DNA per chromosome is 1.2×10^{10} . Therefore when nuclei are isolated at pH 9.0 the DNA is recovered as one molecule per chromosome. As the pH of the solution is decreased, the number of DNA molecules per chromosome increases. Bewer *et al.*⁷ suggested a subunit arrangement of single-stranded units in double-stranded molecules (2.3×10^8 daltons) isolated from slime mould nuclei at pH 7.5. Provided that the strain of slime mould used in our study was not fundamentally different in the organisation of DNA molecules in its

nucleus from the strain used earlier, our results suggest that the 'subunit' arrangement is produced by endonuclease which nicks the DNA at pH 7.5, while nuclear isolation at pH 9.0 allows no such nicking.

When *Physarum* was cultured in the presence of $\text{Hg}(\text{CH}_3)_2$ the S value of its DNA decreased considerably, giving a breakage pattern. In some cases it decreased from the normal 150 at pH 8.0, to approximately 10 at pH 8.0. This indicated that there were as many as 10^5 breaks per chromosome.

To find out whether breakage was independent of replication we labelled the DNA with ^3H -thymidine before the addition of the dimethyl mercury. Figure 1 shows that considerable breakage occurred even in the absence of DNA replication. In another series of experiments we found that damage caused by dimethyl mercury was repaired to some extent after removal of mercury by washing the cells once and resuspending them in fresh medium. After two washings followed by resuspension in fresh medium, repair was nearly complete within 24 h.

To obtain conclusive evidence for repair, we used bromodeoxyuridine (BrdU). A culture of *Physarum* was labelled with ^3H -thymidine in the presence of dimethyl mercury. After 24 h of incubation the cells were washed twice and resuspended in a medium containing unlabelled BrdU. (BrdU is incorporated in to DNA in patches where repair takes place, and was suitable for this experiment because it can be photolysed selectively by ultraviolet light (350 nm), allowing the DNA molecules to be rebroken where the original dimethyl mercury breakage and repair occurred.) When the nuclei of BrdU-treated plasmodia were isolated, part of the isolated DNA was exposed to ultraviolet light at 350 nm, while part of it served as a control. Figure 2 shows conclusive evidence that breakage caused by dimethyl mercury is repaired because the photolysed DNA molecules show the typical breakage pattern, while the molecules unexposed to high wavelength ultraviolet light show the unbroken repair pattern.

It is interesting that dimethyl mercury sensitivity varies from strain to strain of *Physarum*. The strain used in our experiments was a local Ontario strain of *P. polycephalum* while a

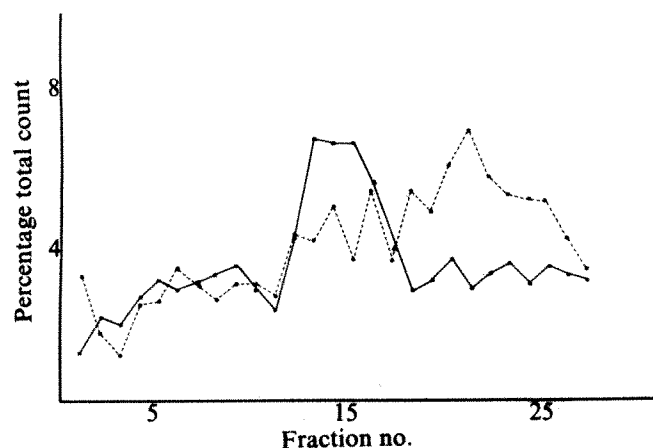


Fig. 1 Sedimentation of $\text{Hg}(\text{CH}_3)_2$ -treated *Physarum* DNA in an alkaline sucrose gradient. Cultures were treated for 24 h with 9.1 mCi ml^{-1} ^3H -thymidine (60 Ci mmol^{-1} methyl ^3H , New England Nuclear). With this treatment the labelled thymidine in medium is exhausted well before the end of the incubation period. At $t = 24 \text{ h}$ $\text{Hg}(\text{CH}_3)_2\text{OH}$ (0.60 mg ml^{-1}) was added to the medium and incubation continued for a further 24 h. Nuclei were then isolated using extraction buffer, pH 8.0. The nuclei were lysed in 0.5 M EDTA 1.0 N NaOH and 1% SDS. After lysis the nuclei for at least 20 min, the DNA was placed on a $5\text{--}15\%$ linear sucrose gradient and centrifuged for 1.5 h at $20,000 \text{ r.p.m.}$ in the SW25.2 rotor of a Spinco ultracentrifuge. Samples (2 ml) were collected from the bottom of the gradient. Albumin ($30 \mu\text{g}$) was added to each tube and the solutions were acidified and precipitated with trichloroacetic acid. The precipitate was collected on glass fibre filters, dried in glass scintillation vials and 5 ml of Omni-Scint (ICN Chemical and Radioisotopes) was added to each vial. Radioactivity was detected using a Beckman liquid scintillation detector. ●, $\text{Hg}(\text{CH}_3)_2$ -treated; ■, control.

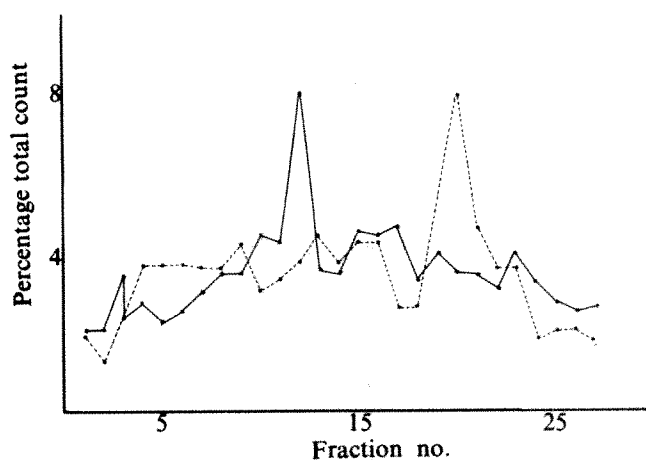


Fig. 2 Evidence for excision repair. Cultures were treated for 24 h in a medium containing ^3H -thymidine ($2.5 \text{ Mi Ci ml}^{-1}$ and $\text{Hg}(\text{CH}_3)_2$. The cultures were washed twice (using low-speed centrifugation) and placed in medium containing BrdU ($20 \mu\text{g ml}^{-1}$), fluorodeoxyuridine ($5 \mu\text{g ml}^{-1}$) and Uridine ($20 \mu\text{g ml}^{-1}$). The cultures were then incubated for a further 24 h. Nuclei were isolated and lysed as described for Fig. 1. Samples (6 ml) of lysate were irradiated in open glass 10-cm Petri dishes for 30 min (350 nm , $26 \mu\text{W cm}^{-2} \times 100$) using a Gelman ultraviolet lamp. Irradiated lysates and unirradiated repaired lysates were analysed in the alkaline sucrose gradient as for Fig. 1. ●, Ultraviolet, (350 nm) irradiated; ■, non-irradiated.

strain of *Physarum* (M_3C) cultured originally at the McArdle Laboratory for Cancer Research in Madison, showed considerable resistance to the effects of dimethyl mercury. Even when the amount of dimethyl mercury present during treatment was double that used in the previous experiments, M_3C showed little or no DNA breakage. If comparable variations in sensitivity to dimethyl mercury-induced DNA damage exist in mammalian populations, the establishment of safe limits of dimethyl mercury in the environment is a more complex problem than might previously have been believed.

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Corrections in the catalogue of oligonucleotides produced by digestion of *Escherichia coli* 16S rRNA with T_1 RNase

SINCE its initial publication¹, the catalogue of oligonucleotides generated by T_1 RNase digestion of *Escherichia coli* 16S RNA has undergone a number of revisions and refinements²⁻⁵. At present the literature contains two rather dissimilar documented versions of this catalogue^{2,3} and disagreements hold for 50% of sequences octamer and larger³. Confusion is not relieved by recent publications concerning the 16S rRNA primary structure^{6,7} that now incorporate some of the oligomer sequences of Uchida *et al.*³ but reject others, without resort to new evidence, however.

The situation demands definitive resolution. The uncertainties regarding oligomer sequences must be settled before one can use comparative approaches to the 16S rRNA sequence and before the ultimate goal of this research, the detailed molecular analysis of ribosome structure and its relationship to function,

can be approached. Therefore, we here review all (T_1) oligomer sequences from the *E. coli* 16S rRNA catalogue for which there has been controversy or uncertainty.

All uncertainties in sequences are herein resolved (with the exception of two post-transcriptionally modified oligomers) due in part to the additional sequence information provided by a new endonucleolytic procedure (unpublished results of M. Sogin, D. S., L. Bonen and C. W.).

All relevant procedures—for generation of oligomers, their separation by two-dimensional electrophoresis, and for their sequence determination—are published in detail elsewhere³. Briefly, in determining individual oligomer sequences, we have used three types of (secondary and tertiary) endonuclease cleavages: (a) pancreatic (specificity for pyrimidines); (b) U2

(preferential cleavage of purines, cleavage of pyrimidines also under more stringent conditions³), and (c) the new activity, T3 (a complex, and so, useful specificity, outlined in Table 1). In almost every instance, a sufficient variety of fragments of an oligomer is generated by these methods to enable a sequence deduced from one subset of fragments to be confirmed by another.

Table 1 presents the correct sequences (and the incorrect versions they replace) for all the oligomers in question. Crucial secondary digestion products used to establish sequence in each case are shown, as are digestion products not found which, by their absence, disprove the incorrect versions.

It has been suggested that many of the differences between the two documented versions of the 16S rRNA catalogue reflect

Table 1 Sequence for the (T_1 nuclease) oligomers in the *E. coli* 16S rRNA catalogue

No.	A	B	C	D
7**	CCUAAACAUG [C(UC)ACAACAUG] (22)	CCUAA, CA, CAUG, CACAUG, UG	CCUAA, CAUG, CACAUG	CAA
17*	AUAACUACUG [AUAACACUUG] (9)	UAA, CUA, CUG, CUACUG	CUA, AUA, CUG	CUUG, CA
21***	CUAAUACCG [CUAAUACG] (25)	CUAA, CUA, UA, CCG, UACCG	CUAAUA, CCG	UACG
33***	AUUAG - 2 copies [AUAUG] (38)	UUA, G	AUU, AG	UA, UG
37**	CUCACCUAG [CUCACCUACAG] (31)	CUCA and CCUA, C(CU)AG	CUCA, CCU, AG, C(CU)AG, CUAG	CA, CAG (T3)
39**	AUCCCUAG [AU(C ₂ U)AG] (29b)	UCCCUA	AU, AG, CCCUAG	
43***	CCACACUG [CACACACUG] (61)	CA, CCA, CUG, CACUG		CCACUG
51***	ACUCCUACG (30b) [uncertain ³]	CG, CUCCUA, CUCCUACG	CG, CU, CCUA	
63*	UUAAUACCUUG [UCUAAUACCUUG] (1) [UUAAUACCUUG] ³	UUAA, UUA, UA, CCUUUG	UUA, AUA, CCUUUG, C(CUU)U, C(CU)U, CUU, UG, UUG	(UCU)A
71*	UACUUUCAG [UACUUCUAG] (7)	UA, CUUUA	CAG, CUUU, UA	CUAG
75***	CCCG* [CCCG] (111)			
89*	CUCAACCUG [CUCAACCUG] (30a)	CUCAA, CCUG	CU, C(CU)G, CUG	CCCUG
105**	CCCCCUG† [CCCCCUG] (93a) ³	CUG, C(CU)G, C(CCU)G	CUG, C ₂ UG, C ₃ UG, C ₄ UG, C ₅ UG, C ₆ U, C ₄ U, and so on	CCCCCCU, CCCCCUG
109***	AUACCCUG [AUACUCCG] (28)	UA, CCCUG	AUA, CUG, C(CU)G, C(CCU)G	CU, CCG
125	ACCCG† (106)			
127**	UUAAAACUCAAUG [UUAAAACUAAUUG] (5)	UUAAAA, etc. UG, CUAAAA, etc.		CUA, UCG
137***	UUUAAUUCG [UAAUUCUUG] (3)	UUUAA, UUCG	UUU, CG	UA, (CU ₄)G
143	ACAUCCACG‡ [ACCAUACG] ‡	CA, UCCA, CG	CAU, ACAU, CCACG(?)	CCA, UCA
161**	CAACCCUUAUCCUUG CAACCCUUUUU (AU, C ₂)G	CAA, CCCUUA, UCCUUUG	CCCUU, CCCUUAU, CCUUUG	UG or (CCU)G, (C ₄ U ₃)A
165	AUAAACUG [AUUAAACG] (21)	UAAA, UAA, UA, CUG		UUA, CG
169**	UCAUCAUG [UCAUCAUG] (14)	UCA, UG, UCAUG	CAUG, UG	UCG
171**	CCCUUACG¶ [CCCCCUUACG] (37) [CCCCUACG] ³	CCCUUA, CG	CG, CCCU, CCU, etc., CCCUU, CCUU, etc.	(C ₂ U ₂)A, CA
175***	CUACACAG C[(AC) ₃ , U]G (55b) [CA, CA, CUA]CG ²	CUA, CA, CG, CACG, CACAG		
179*	CAUACAAAG (50b)	UA, CA	CAU, CAUA, CAA	
181**	ACCUCG [ACUCCG] (75b)	CCUCG	CCU, CG, ACCU	(C ₄ U)G
183**	ACCUCUAAAAG [ACCUCUAAAAG] (19)	UA, UAA, CCUA	CCU, ACCU, CAU, CAUA, AG	(C ₄ U)A

185***	CAACUCG [AAC, U, C]G (65)	CAA, CUCG	CAA, CU, CG	
193***	AUCAG [AUCCAG] (70b)	UCA	CAG, AU	(UCC)A
203***	UACACACCG [U(AG) ₃ , C ₂ G] (56b)	UA, CA, CCG, CACCG, CACACCG		
205***	CC [•] CCG [•] [CCCCG] (101c)			
209**	AUUCAG [AUUUCG] (13)	UUCA, UG	AUU, CAUG, UG	UA, (UUC)G, CG
213**	CUUAACCUUG [CUUAACCUUG] (8)	CUUAA, CCUUCG	CUUAACCUU, CCUU, CG, CCUUCG	UG
223**	AUCACCUCCUUA ^{OH} ** [AUCACCU(CC, U)UA ^{OH}] ³	A, UCA, (C ₄ U ₃)A ^{OH}	CCU, CCUU, CCUUA ^{OH} , AUCACCU	
	CUCG††	UCG	CU, CG	

Correct sequence for the (T₁ nuclease) oligomers in the *E. coli* 16S rRNA catalogue, the sequences of which had previously been disputed, ambiguous or uncertain. ³²P-labelled 16S rRNA isolated from 30S ribosomal subunits of *E. coli* B236 was digested with T₁ RNase and the digest fingerprinted by the two-dimensional electrophoretic procedure of Sanger and co-workers^{3,8}. The first (cellulose acetate) dimension was run in 0.003 M EDTA containing 7 M urea and brought to pH 3.5 with acetic acid; the second dimension (on DEAE cellulose) in 0.1 M pyridinium formate was brought to pH 2.3 with formic acid (unpublished results of M. Sogin, D. S., L. Bonen and C. W.). Individual oligomer spots, located by autoradiography, were sequenced by secondary and tertiary endonucleolytic procedures involving (1) pancreatic nuclease, (2) U2 nuclease, and (3) T3 nuclease activities³. The details of this last procedure will be published elsewhere, but in that it is novel, the procedure will be described briefly here. T3 activity is used chiefly in characterising pyrimidine stretches; it cleaves (T₁) oligomers most readily at U residues, and most slowly at C residues. Thus, for example, the six isomers of (C₂U₂)A would yield the following products, dominant products being overlined: (1) CCUUA → CCUU, CCU, CUU, CUA; (2) CUCUA → CU, CUA, CUCU, (3) CUUCA → CUU, CA, CU; (4) UCCUA → U, CCU, CCUA, CUA, CU, A; (5) UCUCA → U, CU, CA, CUCA; (6) UUCCA → UU, CCA, U, CA. Each correct oligomer sequence (column A) is paired with the incorrect or uncertain versions thereof which have appeared in published versions of the 16S rRNA sequence^{5,6}. The former are assigned numbers designating their relative position in the 16S rRNA sequence^{6,9}; the latter, where appropriate, are given their number designations assigned by Fellner *et al.*³ (*), (**), and (***) indicate respectively that an oligomer of that sequence is found in some other Enterobacteriaceae, most other Enterobacteriaceae, and in over 85% of prokaryotic 16S rRNAs screened^{9,10}. Secondary pancreatic nuclease digestion products, which are in all cases obvious, and concerning which no substantial disagreement exists, are not shown. The important secondary U2 and T3 nuclease digestion products in each case are shown in columns B and C respectively. Their sequences, where necessary, have been proven by further analysis. Column D lists digestion products predicted by the incorrect sequences, but not found. A dot placed over a nucleotide indicates that it has been post-transcriptionally modified. (Superscripts 3 and 7 indicate sequences reported in the corresponding references.)

*This sequence is tentative. Nevertheless, we feel that our data are inconsistent with the projected sequence of Fellner¹³ for these reasons: pancreatic nuclease products are a G, a C, and a spot containing two phosphates, that must be a dimer also by virtue of its electrophoretic mobility on DEAE cellulose in 0.1 M pyridinium acetate, pH 3.5 (PA) buffer; also the dimer must carry positive charge in that it runs faster than AC or CA, (but slower than the slowest monomer, G). T3 cleavage releases the same dimer (it is not cleaved further by pancreatic nuclease) and CG as the major products. For these reasons plus its position on the primary fingerprint³, we believe oligomer No. 75 to be a tetramer, of the form XCCG, where X could be the modified nucleotide previously identified¹³.

†We have detected C₄UG, C₅UG and so on and C₆U, C₄U and so on under conditions where C₆UG or C₆U, had they been present, would have been clearly recognised.

‡ACCCG does not exist in the *E. coli* strain we characterised fully³. But we cannot rule out the possibility that this represents a strain difference. §ACAUCCACG was not reported by us previously³. It appears to have been overlooked, as a minor contaminant in a cluster containing three other oligomers. This sequence has not been seen in other enterobacteria, but the closely related one, ACAUCCAG, occurs in three of the five enterobacteria so far screened¹⁰.

¶We previously reported CCCCU... in this oligomer³, based not on definitive tertiary analysis, but position on the primary fingerprint. T3 digestion proves CCCU and CCCUU to be the largest possible pyrimidine stretches in the sequence.

"We are not certain of the nature, position, and number of the modifications here. We tentatively conclude a sequence C[•]CCG, but cannot rule out the alternative, that is two modified bases, proposed by Fellner¹³.

**In the catalogue of Uchida *et al.*³, the sequence of this 3'-terminal segment had been narrowed to the two possibilities shown. The sequence has also been determined by several other laboratories¹⁴⁻¹⁶.

††Fellner *et al.*³ and Ehresmann *et al.*⁶ report (and place in sequence) four copies of CCUG but no copies of CUCG. Uchida *et al.* found two copies of CUCG and three copies of CCUG in the *E. coli* 16S rRNA catalogue³. We feel this does not represent strain difference. All five enterics screened contain at least one (most contain two) copies of CUCG and three or less copies of CCUG.

strain differences. This explanation we reject in all or almost all instances. Not only are all the oligomers hexamer and larger we report found in both B and K strains of *E. coli*³, but most of them are found in some (*in Table 1) or most (**) of the other Enterobacteriaceae screened, that is *Aerobacter*, *Yersinia*, *Proteus* and *Serratia*, and many of these in at least 85% of all other prokaryotes screened (***) as well⁹⁻¹².

It should be recognised that the oligomer sequence corrections made here do not bring into question the projected 16S rRNA sequence, unless the correction would change the composition of one of the pancreatic nuclease oligomers that overlap a given (T₁ nuclease) oligomer. At present no oligomers for which this latter condition holds are placed unequivocally in the 16S rRNA sequence⁶.

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Note added in proof: Yuki and Brimacombe have recently published¹⁷ evidence inconsistent with three sequences (Nos 143, 165, 175) above, but consistent with the alternative versions we reject. These discrepancies could all reflect errors in interpretation of certain mobilities in the pH 3.5

system, together in one case (No. 143) with 'overcutting' by ribonuclease U2. Specifically, confusion of UUA with CUG (No. 165), UA with CG (also No. 165) and in CUA with CCG (in No. 175) are possible. If this last explanation is correct, it necessitates the interchanging of the oligomers No. 175 (CUACACACG) and No. 203 (UACACACCG) in the 16S rRNA sequence.

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Radioiodine escape is an unexpected source of radioimmunoassay error and chronic low level environmental contamination

PROTEINS and peptides^{1,2} labelled with radioactive iodine (¹²⁵I or ¹³¹I) are commonly used in radioimmunoassays and other investigative procedures. Although decomposition of such labelled molecules can liberate iodide (I⁻) ions^{3,4}, which remain in solution, liberation of elemental radioactive iodine (I₂) has not been reported, as far as we know. Our findings reported here imply that, in conditions common in many radioimmunoassay laboratories, appreciable quantities of gaseous radioactive iodine can indeed escape from radioimmunoassay tubes.

While recounting several radioimmunoassays after a gamma-counter breakdown, we noted an unexpectedly rapid decline of radioactivity in the T tubes (capped 12×75 mm culture tubes containing only 100 µl of the ¹²⁵I-labelled protein antigen solution) which were being used to assess total radioactivity⁵. Because comparable decay was not seen in the other assay tubes, apparent "percentage-binding"^{4,6} seemed to increase progressively. The expected rate of isotopic decay (about 1% per day) was seen with tubes containing only inorganic ¹²⁵I solution, which seemed to rule out isotopic impurity as an explanation.

The possibility that the phenomenon was due to physical migration of isotope was investigated by serial gamma camera scintillography of two specially prepared T tubes. The gamma images (Fig. 1a-c) revealed progressive displacement of isotope from the solutions at the bottoms of the tubes to the tops. The central sections of the tubes remained devoid of detectable radioactivity. During this experiment, total radiation flux remained essentially constant, suggesting that the Parafilm caps had retained the iodine within the tubes, at least during the 2.5 h of observation. Of course, the material at the tops of the tubes would have been virtually undetectable during well-type gamma counting.

To determine whether the losses from the bottoms of such tubes would continue to equal the gains at the tops, several

ordinary T tubes which had already displayed count rate decays of 25% or more were rapidly frozen (to immobilise the solution) and cut in two. The bottoms and tops (inverted) were then counted separately. The count rate for each bottom was essentially the same as for the intact tube. The rates for the tops were significant (2×10^3 - 4×10^3 c.p.m.) but very much less than the missing radioactivity (about 3×10^4 c.p.m.). This finding, together with the apparent absence of radioactivity in the midsections of such tubes (Fig. 1c), implied that a major part of the translocated radioactivity was gaseous (presumably I₂) and had therefore escaped when the tubes were cut. That some of it had escaped even before the tubes were cut became evident when the shield tubes, in which these Parafilm-capped T tubes had been counted initially, also displayed significant radioactive contamination (again, not enough to account for the missing radioactivity).

These initial observations involved a very heavily iodinated preparation of labelled rat follicle stimulating hormone (FSH) (Table 1, line 1), but similar disappearance of radioactivity has been seen with much less strongly iodinated preparations of several pituitary hormones (Table 2). Table 1 contrasts the rapid apparent count rate decays for T tubes with the relatively slower decays displayed by tubes containing (1) various additives in solution; (2) precipitated trace-antibody complex (0-tubes), and (3) a sample of inorganic ¹²⁵I. With each of these labelled hormone preparations, the apparent rate of decay was minimal during storage at 4 °C in screw-cap jars but increased markedly when small volumes (100 µl) were placed in Parafilm-capped culture tubes and kept at room temperature. Accidental or deliberate puncturing of the caps further accelerated the apparent decay rate.

The trace preparations shown in Tables 1 and 2 were all iodinated essentially as described by Midgley⁵, using 0.125-0.75 mCi of ¹²⁵I per µg protein, 15-30 µg of chloramine-T, 60-175 µg of sodium metabisulphite and a reaction time of 40-128 s. About 150 mg of stable potassium iodide was used in the transfer solution. All had been purified by separating iodinated protein from fragments and free iodide, using gel columns (Biogel P-60) pretreated with either 5% ovalbumin or 0.2% gelatin. All had been immediately diluted to working strength in phosphate-buffered saline, pH 7.0, using 0.1% gelatin as the carrier protein.

The use of gelatin, which contains little tyrosine, as an alternative to 0.1% albumin (1% liquid egg white) had been adopted only recently⁷. Before second antibody precipitation, the assay tubes containing precipitates had held larger volumes of 0.1% gelatin solution than the T tubes (500 µl compared

Table 1 Apparent decay (AD) due to radioiodine escape from ¹²⁵I-labelled rat FSH in solution and as antigen-antibody precipitate (0 tubes) and effects of added egg white, gelatin, starch and sodium metabisulphite

Initial specific activity* (µCi µg ⁻¹)	Age of label, day 0	Additive, volume (µl)	c.p.m. (× 10 ⁻³)† and apparent % decay (%AD)						
			Day 0 c.p.m.	Day 2 c.p.m.	%AD	Day 7 c.p.m.	%AD	Day 11 c.p.m.	%AD
275	21 d	—	1,379	1,041	24.5	656	51.5	615	55.5
275	21 d	100% EW, 100	1,404	1,369	2.5	1,248	11.1	967	34.0
275	21 d	1% S, 100	1,401	1,153	17.7	718	48.5	606	56.5
275	21 d	0.1% G, 100	1,395	1,182	15.2	759	46.3	617	55.7
275	21 d	10% EW, 500	1,380	1,350	2.2	1,276	7.5	1,213	12.1‡
275	21 d	1% S, 500	1,390	1,343	3.4	1,235	11.2	1,140	17.9
275	21 d	0.1% G, 500	1,388	1,340	3.5	1,221	12.0	1,113	18.7
275	30 d	—	1,212	940	22.4	628	48.0	—	—
275	30 d	0.2mg M, 100	1,242	1,182	4.8	962	22.5	—	—
188	3 d	—	670	613	8.5	488	28.0	457	31.8
188	12 d	0 Tubes (n=6)	134 ±0.85	130 ±0.97	3.0	116 ±1.13	13.4	109 ±0.39	18.7
Inorganic ¹²⁵ I			2,730	2,670	2.2	2,521	7.7	2,387	12.5

The distinctly overiodinated preparations (initially about 2.38 and 1.63 atoms ¹²⁵I per molecule, respectively) were prepared on February 28 and March 18, 1975 and, except for the 0 tubes and metabisulphite experiment, studied concurrently. Effects of adding EW, G and S were similar with both preparations. The rat FSH (rFSH), and the rat LH, TSH and prolactin (rLH, rTSH and rProl) shown in Table 2, were gifts from the NIAMD rat pituitary hormone distribution program. EW, egg white; G, gelatin; S, starch; M, metabisulphite.

*Expressed as µCi in protein peak per µg protein iodinated.

†All counts are in hundreds, that is, 1,379 = 137,900 c.p.m. Each datum (except for 0 tubes) is the mean of duplicate tubes.

‡Note complete prevention of ¹²⁵I escape during 11 d at room temperature.

Table 2 Apparent radioactivity decay in ^{125}I -labelled pituitary hormones of high and low initial specific activities

Labelled hormone	Initial specific activity* ($\mu\text{Ci } \mu\text{g}^{-1}$)	Age of label day 0	c.p.m. ($\times 10^{-2}$)† and apparent % decay (%AD)				
			Day 0		Day 4		Day 7
			c.p.m.	%AD	c.p.m.	%AD	c.p.m.
rFSH	63	< 2 h	846	765	9.6	710	16.1
rLH	400	17 d	939	650	30.8	530	43.6
rLH	62	< 2 h	677	609	10.0	577	14.8
oLH	230	15 d	1,120	996	11.1	906	19.1
rTSH	279	15 d	1,194	908	24.0	771	35.4
rTSH	80	< 2 h	984	862	12.4	798	18.9
rProl	85	2 h	894	792	11.4	753	15.8
Inorganic ^{125}I			2,730	2,670	2.2	2,521	7.7

See legend of Table 1 for identities of rat hormones. The preparation oLH is LER-1056 C2, a highly purified ovine luteinising hormone given by Dr L. Reichert.

*See first footnote, Table 1.

†See second footnote, Table 1.

with 100 μl) as well as other proteins (immune and non-immune sera) not present in the T tubes. Table 1 shows that the presence of protein solution (even gelatin, in adequate volume) can retard, or even totally prevent, radioiodine escape.

A complete explanation of this phenomenon is beyond the scope of this report. There are two possibilities. One is that iodine can be released from covalent bonding to tyrosine residues as a result of radiation damage³. The other is that it is released from non-covalent radioiodine-protein bonds. It is known (Fig. 8b and c of Yalow and Berson³) that free radioiodide does appear on storage of ^{125}I -labelled protein preparations. This radioiodide may be converted, at least in part, to radioiodine. The facts that (1) escape of radioiodine accelerates when trace solutions are brought to room temperature and exposed to air, and (2) addition of metabisulphite can partially prevent this escape (Table 1) suggest that some type of chemical oxidation is involved.

Escape of radioiodine poses two sets of problems. The most immediate is the potential hazard to laboratory personnel who may be undergoing chronic, low level exposure to an environment contaminated with radioiodine. The extent of this hazard would be expected to vary widely, because of variations in rates of trace protein decomposition (Tables 1 and 2) and amounts of radioiodine-labelled protein subjected simultaneously to destabilising conditions. Thyroid scans of the four individuals who would have received the greatest exposure during the 2-month period throughout which ^{125}I had been escaping in our laboratory (that is before the institution of the precautions discussed below) revealed no detectable

neck radioactivity (less than 0.05 μCi). Conditions during this short period (moderate numbers of assay tubes, high rate of air exchange) did not favour maximum environmental contamination. We have, however, calculated that laboratory conditions could exist in which air concentrations of radioiodine escaping from assay tubes could exceed US Nuclear Regulatory Commission air concentration limits (10 CFR 20, Appendix B) by as much as 500 times. (To reach these extreme limits, large numbers of assay tubes would have to be kept at room temperature in a small space with a poor rate of air exchange. Nevertheless, since this report was prepared, we have learned of one laboratory in which detectable neck radioactivity was encountered in a technician who worked in a room in which discarded assay tubes were stored.)

Radioiodine escape, at any rate, is clearly undesirable⁸, particularly with ^{125}I (half life 60 d). Allowing it to occur unchecked now seems inexcusable, since it can be minimised by the following simple precautions: adding a tyrosine-rich carrier protein (such as albumin) to the solution in which trace is stored; keeping stored solutions tightly capped and cold; adding a sufficient volume of albumin solution to all assay tubes. In the important case of the predominant assay tubes, those containing antigen-antibody precipitate, the small but distinct decay due to iodine escape during storage at room temperature (0-tubes in Table 1) can be blocked for at least 21 d by covering the precipitates with 500 μl of 1% albumin solution immediately after decanting the supernatant. (This innovation has noticeably improved assay precision in our laboratory). These precautions, however, would not be expected to prevent protein-radioiodine dissociation, but only to conceal its development by trapping the isotope in solution, as ^{125}I -labelled albumin. Progressive distortion of measurements of antigen-antibody interaction^{4,6} would still result. Nonspecific labelling and/or label lability could also complicate tissue localisation studies⁹.

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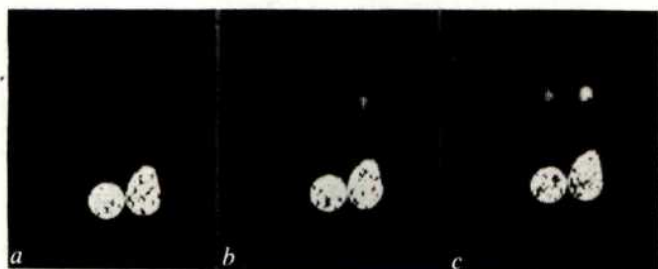


Fig. 1 Successive gamma camera images of two Parafilm-sealed (12×75 mm) culture tubes containing ^{125}I -labelled rat FSH solution, showing progressive saltatory displacement of ^{125}I from solution. Times: (a) 15-35; (b) 75-95; (c) 135-155 min after preparation. The gamma camera was a Searle Pho-Gamma HP coupled to an Intertechnique Cine 200 Data Collection System. Each tube contained 0.1 ml of a concentrated solution of the ^{125}I -labelled FSH shown on line 1, Table 1, and was sealed with three or four layers of Parafilm. About 15 min after preparation, the tubes were mounted upright in the field of the (pinhole-columnated) gamma camera. Although they were not moved during the series of 20 min exposures, the tube on the right had been shaken manually (before mounting) to simulate travel in an automatic gamma counter. This seemed to accelerate subsequent volatilisation of radioactivity. The use of concentrated solution was dictated by gamma camera availability. Similar pictures of ordinary T tubes would have required longer exposure periods.

Erratum

In the article "Methyl group as a probe of chirality in Raman optical activity" (*Nature*, **255**, 458; 1975) the author's name should be L. D. Barron and not as printed. Parts of the figures in the original article could not be seen clearly so they are reprinted below on a larger scale.

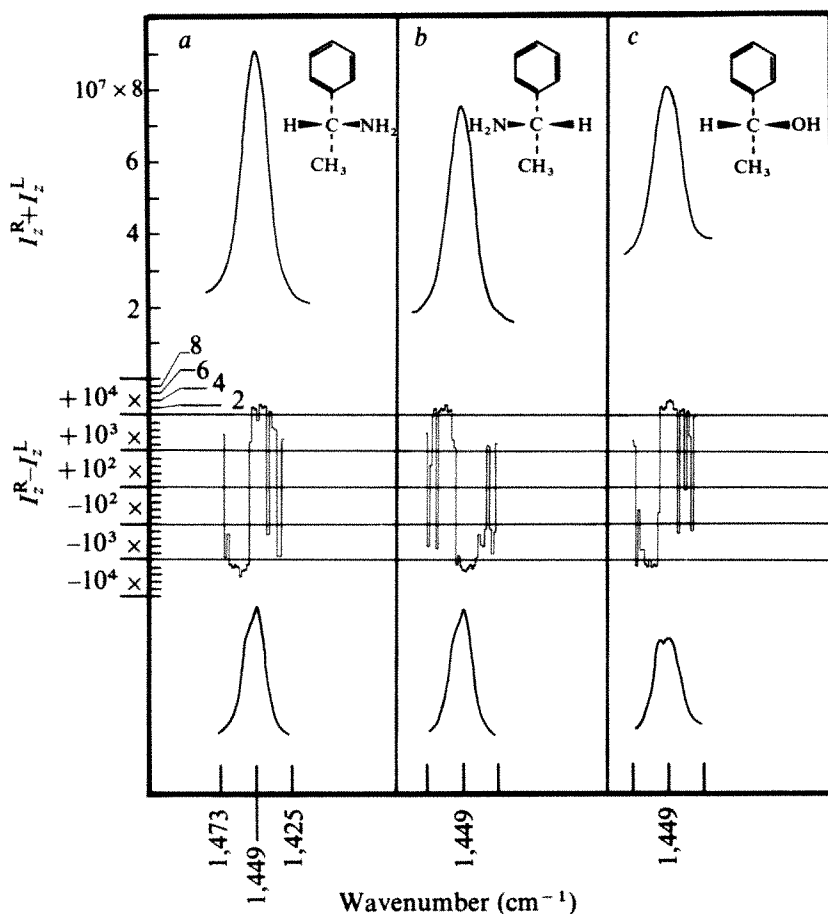


Fig. 1 The depolarised Raman circular intensity sum (top) and difference (centre) spectra, as recorded, of methyl asymmetric deformations in: *a*, (+)- α -phenylethylamine; *b*, (-)- α -phenylethylamine; *c*, (+)- α -phenylethanol. At the bottom is a sum spectrum at high resolution. Instrumental conditions: laser wavelength 4,880 Å, laser power 1 W, slit width 1,500 μ m for the top and centre spectra and 200 μ m for the bottom spectra. The absolute configurations are taken from ref. 10.

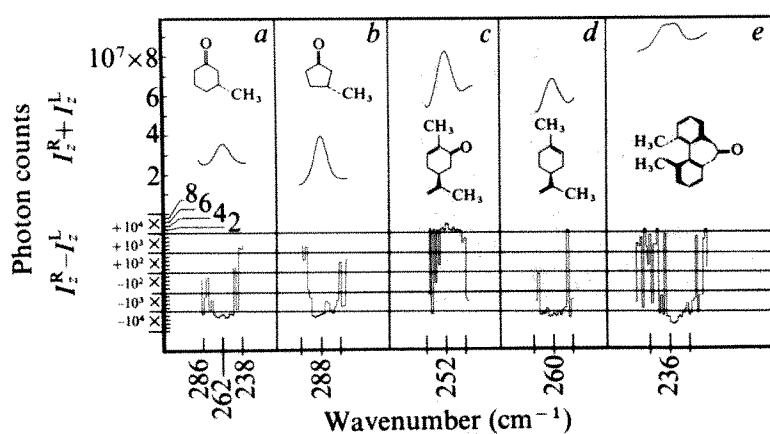


Fig. 2 The depolarised Raman circular intensity sum and difference spectra of methyl torsions in: *a*, (+)-3-methylcyclohexanone; *b*, (+)-3-methylcyclopentanone; *c*, (+)-carvone; *d*, (-)-limonene; *e*, a saturated solution of (+)-dimethyldibenz-1,3-cycloheptadiene-6-one in acetone. Instrumental conditions as for the top two spectra in Fig. 1, except that a laser wavelength of 5,145 Å was used in *e* to minimise fluorescence.

reviews

THIS volume* contains 18 papers presented in 1972 at a conference attended by scientists, historians and philosophers at the Villa Serbelloni in Bellagio, Italy. Each paper is followed by comments of one or more of the participants, usually Bernard Rensch. If the subject of this conference was, as Ledyard Stebbins (p. 285) claims, reduction in biology, then few of the participants addressed themselves to the issue.

The two boundaries between natural phenomena which are of greatest interest in arguments over reduction are that between the mind and physiological processes and that between biological processes in general and the subject matter of physics and chemistry. Until quite recently, all of the carefully worked out examples of reduction have concerned reduction within physics, the reduction of one physical theory to another. Thus, the next step would seem to be the analysis of similar examples within biology. Furthermore, these are exactly the examples that professional biologists are best prepared to deal with constructively. But the topic never arose. Instead, most of the papers which touched on reduction at all, dealt with the reduction of mind to physiological processes and a few concerned the reduction of biological processes to physics and chemistry.

The papers divide fairly naturally into three groups: historical, biological, and philosophical. The philosophical papers divide somewhat less easily into those which are straightforward philosophy and those which are 'philosophical' in the pejorative sense. The papers by Ernest Boesinger, June Goodfield, and G. Montalenti are primarily historical. Boesinger traces the fate of the evolutionary hypothesis after Lamarck and Darwin, giving special attention to the bizarre situation which still prevails in France. Goodfield attempts to derive some conclusions about reductive strategies by studying the methods and results of a variety of biologists in the 19th and 20th centuries. Montalenti compares mechanistic reductionism and holistic vitalism in Democritus, Aristotle, and Darwin. Gerald Edelman's paper is also historical, discussing as he does the recent history of the theory of clonal

Philosophy of reductionism in biology



Aristotle—mechanistic reductionism and holistic vitalism. Taken from Hartmann Schedel Liber Chronicarum (Nuremberg Chronicle), Nurnberg, 1493.

selection in antibody formation. The papers by Dobzhansky, Stebbins and John C. Eccles are mainly biological. If there was ever an excuse for the ill-informed parodies that so often pass for discussions of the synthetic theory of evolution, Dobzhansky's lucid synopsis eliminates it once and for all.

The papers by Morton Beckner, Dudley Shapere, Francisco Ayala, Donald Campbell, and Karl Popper are truly philosophical. Beckner exploits the distinction between hierarchically arranged theories and hierarchically organised natural systems. Shapere expands upon the difference between compositional and evolutionary theories and the different sorts of problems which give rise to them. Donald Campbell sets out his views on evolutionary epistemology, views shared in part by Popper and Henryk Skolimowski. Just as biological species adapt to their changing environments by a process of variation and selective retention, individual organisms come to learn about their environments and successive scientific theories come to characterise the real world with ever increasing accuracy. One problem with this

philosophical thesis concerns the appropriate adjective required to modify the term 'variation'. In what sense are mutations 'chance'? In what sense is learning and the development of science 'blind'?

Ayala presents a surprisingly informative and sensitive linguistic analysis of "evolutionary progress", surprising because such analyses are rarely informative and even more rarely produced by practicing scientists. Stebbins also deals with the notion of progress in his contribution. Peter Medawar presents an uncontroversial comparison between levels of generality in geometry and the natural sciences. Skolimowski, on the other hand, presents an unnecessarily petulant attack on the conventional notion of rationality in science. The tone of Skolimowski's paper is especially unfortunate because the issues are both important and inherently liable to distortion and caricature.

Large chunks of the papers by Rensch, Eccles, W. H. Thorpe, and Charles Birch exemplify what can happen when a noted scientist tries his hand at 'philosophising': these papers are uncomfortable reading. The biology is accurate and often inherently fascinating—for example, Eccles' discussion of split brain experiments and Thorpe's description of migration and exploratory learning—but too often their attempts to set out philosophical theses are as embarrassing as the efforts of an ageing *diva* trying to sing the latest pop song. Although Shapere values interdisciplinary endeavours, he replies to these poorly formulated views in the only way a professional philosopher can (pp. 256, 258). Just as scientists are entitled to establish standards of competence for their undertakings, philosophers have a right to expect at least minimal competence in theirs.

I have, however, saved the best part of the book until last. Monod did not present a formal paper at the conference; instead, he defended his book *Chance and Necessity*. The ensuing discussion points up, in the most direct manner possible, both the obscurity and importance of the problems which surround the issue of reduction. Several of the papers in this volume go a little way towards reducing the obscurity, and the emotional exchange between Monod and Skolimowski attests to the importance of these issues. I could not recommend that anyone read this volume cover to cover, but a few of the papers are worthwhile and the final 20 pages, in which the participants quiz Monod, should not be missed.

David L. Hull

**Studies in the Philosophy of Biology: Reduction and Related Problems*. Edited by Francisco J. Ayala and Theodosius Dobzhansky. Pp xix+390. (Macmillan: London and Basingstoke, September 1974.) £12.00.

THIS volume* is the result of a gathering in April 1974 of scientists from different disciplines with a common interest in the thymus. Fifty-three contributions on various aspects of the thymus unfortunately make heavy reading and because they are all formal papers it is difficult to measure the success of the meeting in providing a forum for the exchange of ideas. Rarely does one find proceedings that do justice to the tone and atmosphere of a meeting.

Although all immunologists have believed in the importance of the thymus, most in the past have paid only lip-service to the notion that thymocytes and T cells exert their influence through soluble factors. In the last four years, however, this has changed and now everybody is chasing factors. The inevitable result has been an explosion in the literature and a proliferation of factors which reflects more the individuality of the scientists than the molecular nature of the factors themselves. To a certain extent this book provides an opportunity to compare the many different approaches of the workers in the field, but this could have been made easier by a more extensive editorial summary.

The volume is divided into six sections, by far the most readable of which are the first (the T cell story) and the last (perspectives of the role of thymus factors in immunity), which can be recommended to any reader. The filling of this sandwich comprises sections on soluble factors and T cell development, the preparation of thymic factors, their activity *in vitro* and their activity *in vivo*. The articles are generally well presented and illustrated but many of course have been superseded in the year it has taken to publish this work. This is a universal fault with conference proceedings and it is a shame that it has happened in this case because this is a very useful reference work. Although it contains much on thymic hormones, it has little to say about the functioning of immune responsiveness genes—which is a pity as this is a fascinating and rapidly expanding area.

What the volume does achieve is to highlight the lack of standardisation of thymic factors. One may hope that an attempt to achieve agreement in this area will arise out of meetings such as this. Another salutary achievement is

Role of the thymus in immunobiology

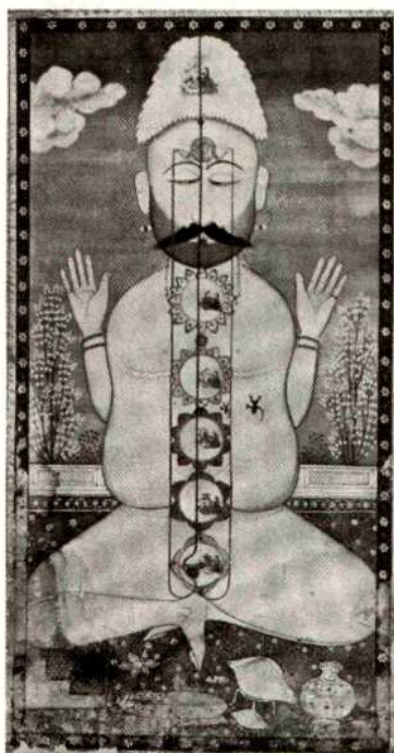


Diagram of the six chakras in the subtle body, Kangra, Himachel Pradesh. Gouache on paper, eighteenth century. "The first and fundamental image which reflects inner facts is the 'subtle body', which is shared by all Indian traditions, as well as by Indian medicine. It is experienced as that immense ramification of channels of energy which flows through the entire body." Taken from *The Body as a Medium of Expression* edited by Jonathan Benthall and Ted Polhemus. Pp. viii + 333. (Allen Lane, Penguin Books: London, May 1975.) Hardcover £7.00; paper £3.50.

in the underlying current of caution expressed in interpreting cellular function by the presence or absence of differentiation antigens on cells exposed to thymus factors.

Norman A. Staines

SINCE the discovery in 1968 of genetic athymia in 'nude' (*nu/nu*) mice, the continuing study of these animals is throwing new light on the functions of the mammalian thymus. About 200 papers have been published so far, and an analysis of the present state of the subject would obviously be very useful.

Dr Rygaard's book† does not satisfy this need adequately. It was, in fact, published in Denmark in 1973 and is now being marketed more widely, without change or addition. Unavoidably, in a fast moving field it is already out of date. Suffice it to say that the majority of research reports on the nude mouse belong to the period after the book was written. Furthermore, out of 83 papers known to me from the period up until the end of 1972, 34 are not listed in the references.

A quarter of the book is devoted to the author's own line of experimentation—demonstrating that the athymic animal accepts grafts from a variety of vertebrate species. Data of body and organ weights of nude and control mice are presented in convenient tables. Rather unexpectedly, spleen and lymph node weights were usually higher in nude mice than in normal controls. The levels of various serum proteins, including gamma globulins, were also normal. The problems raised by some of these data are pointed out but not followed up.

The coverage of other topics is rather uneven and cursory. No tables, graphs or illustrations from other workers' original reports are incorporated. Work on the production of humoral antibodies is summarised rather too briefly.

The last two chapters are based on the author's own finding that no spontaneous tumours developed in any of over 11,000 nude mice recorded. He rightly points out that the short life span of these animals permitted an average observation time of only 4 months, but proceeds to deemphasise this difficulty on grounds that are not altogether decisive. The statement, for example, that auto-antibody production is rare in nudes can be questioned. Essentially, on these bases, the author expresses doubts about the theory of immunological surveillance and declares his conversion to the ranks of agnostics in this respect.

The book is written in a lively style and is attractively produced. In spite of its limitations, it is likely to stimulate interest in the study, through the nude mouse mutation, of the thymus. E. M. Pantelouris

**Thymus Factors in Immunity*. (Annals of the New York Academy of Science, Vol. 249.) Edited by Herman Friedman. Pp. 547. (New York Academy of Sciences, February 1975.) \$39.00.

Corrigendum. In the review of *Registry of Mass Spectral Data: Volumes 1-4* (*Nature*, 257, September 4, 75, 1975), the collective price of the four volumes was incorrectly stated as £250.00. The price should have read £155.25.

†*Thymus and Self: Immunobiology of the Mouse Mutant Nude*. By Jørgen Rygaard. Pp. 193. (Wiley-Interscience: London and New York, March 1975.) £6.50.

Mechanics of Polymers. By R. G. C. Arridge. Pp. ix+246. (Clarendon: Oxford; Oxford University: London, June 1975.) £6.50.

Books on the mechanical properties of polymers range between two extremes: the practical approach is to present large quantities of data held together with theoretical interludes, whereas the pure approach is to stick mainly to theory and only introduce data occasionally.

This book is predominantly of the second sort. There is a thorough discussion of elasticity theory, including large strain elasticity and anisotropy, and a chapter on time-dependent elasticity theory, but these are left hanging as the chapter on applications to polymers discusses mainly transition temperatures and test methods.

The treatments of anisotropy and of yield and fracture are more integrated, although there are relatively few examples. The discussion of fracture is very short.

This book is too uneven to be a good general text, although individual chapters may prove valuable. Most of the material is also in Ward's *Mechanical Properties of Solid Polymers* (1971). An understanding of vectors and tensors is required. Most of the time SI units are used. Some of the diagrams, particularly of chemical structures, are unclear.

P. D. Calvert

Applications of High-speed Liquid Chromatography. By J. N. Done, J. H. Knox and J. Loheac. Pp. vii+238. (Wiley-Interscience: London and New York, January 1975.) £6.50.

THE technique of high speed, high pressure, liquid chromatography (HPLC) is one of the more rapidly expanding areas of analytical chemistry: it now rivals gas chromatography in terms of practical importance. The primary advantage of the method is that it can separate compounds which are not readily amenable to gas chromatography; for example, polymers, high molecular weight compounds, and ionic and strongly polar species such as steroids and sugars, and so on.

In the modern analytical laboratory any decision to invest in a new technique is often dependent on an assurance that it can solve a specific problem. This book aims to assist in this decision and also to provide a much needed practical guide to the field.

After a brief but effective coverage of the technique and theory of high speed liquid chromatography the major section of the book consists of examples of selected chromatograms taken from the recent literature. The 150 examples

shown illustrate the wide range of compound types amenable to HPLC and cover hydrocarbons and petroleum, acids, amines and nitrogen compounds, phenols and antioxidants, sulphonic acids and dyestuffs, insecticides and herbicides, nucleotides and nucleic acid bases, pharmaceuticals, steroids and natural products. Each chromatogram includes details of column material, column length, packing and particle size, operating temperature, pressure type of detection and sensitivity.

As the authors correctly point out, the rapid advances in this field will lead to improvement in some of the chromatograms but the operating conditions will not undergo any radical changes.

Altogether this is an excellent book, concise and well documented and providing the essential information for anyone contemplating the use of this important technique.

B. Fleet

Books brief

Compaction of Coarse-grained Sediments, (Developments in Sedimentology 18A.) Edited by George V. Chilingarian and Karl H. Wolf. Pp. 552. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl. 130; \$54.25.

THIS latest contribution to the series *Developments in Sedimentology* is the first of a two-volume appraisal of compaction of coarse-grained sediments, and complements an earlier volume dealing similarly with argillaceous sediments. For such a highly specialised subject, embracing so many disciplines, the editors have succeeded in assembling a balanced collection of well presented and illustrated papers which should largely satisfy geologists and engineers working in this field. Most of the chapters are essentially of a review nature and contain comprehensive bibliographies; as such, the book should serve as an invaluable source of reference.

Of specific interest to geomechanics are chapters dealing with experimentation and mechanics of sand compaction (Allen and Chilingarian), its effects on such properties as sediment porosity, permeability, strength and so on (Ingles and Grant), and a detailed presentation of mathematical models illustrating problems arising from the compaction of porous solids (Raghavan and Miller).

Sedimentologists are catered for in papers dealing with compaction of carbonate sands (Coogan and Manus), a case study demonstrating how

differential sand-mud compaction can determine the geometry and distribution of elongate sandstone bodies (Brown) and the use of geophysical well logs in recognising depositional environments and sediment compaction (Allen). Bissell and Chilingarian review the magnitude and possible causes of hypersubside in various sedimentary basins in North America as well as reporting on localised subsidence related to oil and water extraction.

The second part is eagerly awaited.

Brian Waugh

Chemical Oceanography. Edited by J. P. Riley and G. Skirrow. Volume 1. Pp. xx+606. £18.50; \$49.00. Volume 2. Pp. xx+647. £19.50; \$51.50. (Academic: London and New York, June 1975.)

THIS edition up-dates the previous one of about a decade ago and these volumes benefit from more quantitative descriptions of marine chemical phenomena. Refreshingly, an attempt has been made to standardise units of measurement throughout these volumes. Most of the chapters contain a complete range from basic theory to modern applications in the field. An excellent example is the chapter on seawater as an electrolytic solution, in which well-developed arguments are used to explain its complex electrolytic nature. The overview that this and some other chapters present is the most significant improvement on the earlier edition—frequently a catalogue of random measurements of chemical species.

The impression that chemical oceanography lags some way behind other fields of science is dispelled by many of the chapters. Others only confirm this impression—for example, the essay on micronutrient elements comprises much work completed twenty years and more ago. The chapter on dissolved organic material in seawater confirmed the unsatisfactory state of research in this area. The all-embracing expression 'dissolved organic matter (DOM)' appeared too often and is of limited usefulness. The inclusion of DDT and PCB seemed out of place, and I was not convinced as to the accuracy or completeness of the information presented on chlorinated hydrocarbons.

The opportunities for criticism in these volumes were few. The books taken as a whole provide a comprehensive picture of chemical oceanography today. To anyone interested in the marine environment I warmly recommend this major work.

M. M. Rhead

obituary

William Marshall Smart, FRSE, FRAS, Regius Professor of Astronomy in the University of Glasgow from 1937–59, died on September 17 at the age of 86.

Professor Smart was born in Scotland and gained his MA at Glasgow in mathematics and natural philosophy in 1910 and his BSc in 1911. He was awarded a scholarship to Trinity College, Cambridge, where he read for the mathematical tripos. After World War I, which he spent in the Royal Navy, he returned to Cambridge as John Couch Adams Astronomer and Lecturer in Mathematics. He was the author of several popular books on astronomy (*The Sun, The Stars and the Universe*, 1928, *Stellar Dynamics*, 1938, and *Some Famous Stars*, 1950) and also published textbooks on the mathematical theory of astronomy. During World War II, he returned to the problems of navigation, publishing

several books which were to play a significant role in the training of navigators in the Navy and Air Force. He was President of the Royal Astronomical Society and a Vice-President of the Royal Society of Edinburgh.

Marshall Kay, Newberry Professor Emeritus of Geology at Columbia University, died on September 3 in New Jersey at the age of 70.

Born in Ontario, Dr Kay gained his first degree in 1924 at the University of Iowa and his PhD at Columbia in 1929. He joined the geology faculty as a lecturer and became assistant professor in 1937, an associate professor in 1941 and a full professor in 1944. In 1967, he became the sixth Newberry Professor of Geology. Dr Kay paved the way for new global tectonics with

his pioneering modern continental drift theory. His theories from reconstructions of continental movements, demonstrating that North America's boundaries were delineated 400 Myr ago when upheavals in the ocean floor consolidated offshore chains of volcanic islands with the mainland, supplanted earlier theories of the origin on the North American continent. Dr Kay suggested that on the basis of his theory of expanding continents, Japan would eventually merge with the Asian mainland. The theory also envisages Alaska again becoming part of Siberia and that the China Sea would 'disappear' into South Asia. "For profoundly influencing the theory and practice of modern stratigraphy", the Geological Society of America presented him with the Penrose Medal. He was also awarded the Kunz Prize by the New York Academy of Sciences.

announcements

Award

The **Gold Medal** of the Canadian Association of Physicists has been awarded to **J. A. Jacobs**.

Appointments

J. L. Links and **J. C. Polkinghorne** have been appointed members of the Science Research Council.

A. J. Buller, **A. P. M. Forrest**, **H. Kay** and **C. M. S. Saunders** have been appointed to the Medical Research Council. **Sir John Gray**, present Secretary to the Council, is to become Deputy Chairman.

International meetings

October 4, **Analytical aspects of ozone and odor control with ozone**, Miami (International Ozone Institute, Skytop Complex, Syracuse University, Merrill Lane, Syracuse, New York 13210, US).

October 14, **Scientific results from the Ariel-5 satellite**, London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

Person to person

Paris-Reading. Two bedroomed house wanted in or near Paris, convenient for the University of Paris VII, Place Jussieu, for one year from January 1976, possibly in exchange for house in Reading (Dr J. Walker, Physics Department, Reading University, Whiteknights, Reading RG6 2AF, UK; Reading 85123 ext. 388).

Paris-New York. French biochemist, wife and child seek four-room furnished apartment in New York City in exchange for similar accommodation in central Paris for a sabbatical year starting in September 1976 (Dr D. Barritault, 4, rue Française, 75001 Paris, France).

There will be no charge for this service. Send items (not more than 60 words) to Holly Connell at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

October 21–23, **War on waste**, Warwickshire (Exhibition Managements Ltd, 159 Mortlake Road, Kew, Surrey, UK).

November 13–14, **Water structure and transport in biology**, London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

November 17–20, **Juvenility in woody perennials**, Berlin (Professor A. Karnatz, Institut für Nutzpflanzenforschung Obstbau, T V Berlin, Fachbereich 15 D-1000, Berlin, Dahlen Albrecht Thaweg 3, Germany Fed Rep).

November 19–21, **Nuclear science**, San Francisco (R. S. Larsen, SLAC, Stanford University, PO Box 4349, Stanford, CA 94305).

November 24–26, **Medcomp**, Berlin (AMK, c/o Online Conferences Ltd., Cleveland Road, Uxbridge, UK).

November 29–December 3, **Alcohol and drug dependence**, Bahrain, Arabian Gulf (International Council on Alcohol and Addictions, Box 140, 1001, Lausanne, Switzerland).

nature

October 9, 1975

Postgraduate education: MITs in Britain?

ON the same day that the Science Research Council (SRC) was launching its annual report (bearing, predictably, little good news for the practitioners of 'big science') it was also publishing a much less predictable document that makes some very radical suggestions. *Postgraduate Training* (SRC, free) should be read by every postgraduate, every academic and every industrialist in the business of hiring university-educated people.

A year and a half ago, the Commons Expenditure Committee looked at the postgraduate training scene in Britain across a spectrum from student funding to national relevance. It took advice from a strangely limited range of witnesses and came up with a dim view of the utility of higher degrees. The Department of Education and Science has been slow to reply to the criticism; in a sense this report can be seen as an attempt to remedy that deficiency. In no way, however, is it a stonewall defence of the *status quo*. The SRC working party, chaired by Sir Sam Edwards, has instead taken upon itself to inject ideas for change into the debate, and to propose major reorganisation at the postgraduate level.

The guiding principle behind the report is that postgraduate education should become less of a training ground for research workers and more of a broad continuation of the educational process. Several reasons are advanced. One is that the working party sees many scientific disciplines as having established a broad enough base to support technology "for a considerable time", and so there is now a greater need to train for careers for outside research. This point will cause alarm to some who will see it as the thin end of the wedge by which research, particularly in the physical sciences, is to be gently downgraded. Another reason for changing the emphasis of postgraduate training is to help persuade industry to take high qualifications more seriously. The working party has some hard words for the 'watching old Tom' school in which the sooner bright young students are out of university and learning the job by personal experience the better. A third reason, implicit in much that the working party proposes, is that there is a totally unsatisfactory and wasteful relationship between universities and professional bodies. It is farcical that in 1975 the working party has to suggest that the SRC enquire of these bodies whether certain university and polytechnic courses might be approved for professional training schemes. Fourth, even those who are ultimately to stay in research need education far beyond a first degree if they are not to become narrowly specialised. The provision of such education is at present patchy; many PhD students are able to avoid, or are unable to obtain, any formal training from the day they graduate.

The main recommendations are blunt.

- New, broader degrees to be available, both at master's and doctoral level, in which taught courses have an enhanced significance.
- A higher proportion of compulsory course work to be included in the first year of PhD courses.
- The option of proceeding to a PhD to be open only to those who have taken course work and in addition show aptitude for research.
- Consortia of universities, polytechnics and non-academic bodies to be formed to develop wider ranging courses.

If this looks much closer to the American pattern of postgraduate education, this is by design. The Edwards report is a curious echo of the Flowers report on university computer needs exactly 10 years ago. Then it was IBM which was the object of much admiration; now it is MIT. "We are anxious", says the report, "that the opportunities for graduate instruction offered by MIT should be matched by consortia of British universities, if not by individual universities."

And then finally, lest any academic applegarts be left un-upset, the working party recommend that postgraduates be paid differentially on the basis of the extent to which their work is of economic importance or in the national interest. Since the giving of grants is basically an SRC concern, such a measure could be rapidly implemented, although it is obvious that it could not be applied fairly except in some rather limited but clearly defined instances particularly favoured by SRC, such as joint SRC/SSRC studentships, total technology studentships and the Co-operative Awards in Science and Engineering (CASE) scheme.

The overall impression left by the report is certainly exciting and progressive, and it will speak directly to all those who know and approve of American higher education. But it all reads a little like a lecture, albeit a very good one, prepared by half-a-dozen lively minds gathered round a dinner table. Can the ideas be carried further than oratory? Is there any evidence, for instance, that the disappointing response to the CASE scheme would not be repeated on a much larger scale? Is there any indication that industry is prepared to accept more highly trained entrants? Do people say bad things about MIT's postgraduate education? Is a broad postgraduate training any use without broader undergraduate training than we have now?

That the working party is serious about its proposals, however, is in no doubt. "At a time when we are having to urge belt-tightening on everyone", says Sir Sam, "we wouldn't be proposing something as expensive as this were we not in deadly earnest". □



Nuclear fuel monitoring array at the Fast Flux Test Facility, Hanford, Washington

Rough passage in USA for first breeder reactor

The United States government is planning to spend more than \$10,000 million to develop a liquid metal fast breeder reactor. The programme is the highest priority energy research and development effort supported by public funds, but it has recently come under heavy criticism and it is beset by technical problems. In the second of a series of three articles, Colin Norman reports.

TO its supporters, who include President Ford and his predecessor, the liquid metal fast breeder reactor (LMFBR) could mean the difference between long term prosperity and economic disaster for the United States. But to its critics, who are legion, the reactor is an unacceptable health hazard and an unconscionable waste of taxpayers' money. The debate between those two camps, which has been reverberating for several years, has been getting more shrill lately, but about the only sure fact to emerge so far is that the federal government's costly effort to develop a commercially viable LMFBR is in trouble.

Two separate attempts to shut off funds for key elements in the LMFBR

programme were made in Congress this year. Although they were defeated, similar moves can be expected next year, when the programme's budget will be an even plumper target. Meanwhile, three Congressional committees have been studying the rationale for the programme, individual Congressmen have been taking well publicised pot shots at it, the General Accounting Office has recently issued a gloomy assessment of the effort's cost and timing, and a committee of the National Academy of Sciences is examining the project as part of a mammoth investigation of the entire nuclear power programme in the United States.

It is not difficult to pinpoint the reasons why the LMFBR programme has attracted so much controversy. For a start, it is the single most expensive energy research and development project supported by the federal government; it has also experienced huge cost overruns, has fallen badly behind schedule, and has encountered some nagging technical and safety problems.

But an equally important reason why the LMFBR has come under such concentrated hostile fire is that it is the centrepiece of the Administration's long term nuclear power programme. Without a commercial breeder reactor, the argument goes, light water reactors will run out of low-cost uranium within a few decades, but the LMFBR could prolong the use of nuclear fission as an energy source for several centuries. In other words, if the LMFBR is halted, the nuclear power programme itself will eventually be curtailed, which is reason enough for the breeder reactor programme to have become something of a rallying point for various environmentalist and anti-nuclear groups. Underlying the debate about the need for the LMFBR is a fundamental divergence of opinion on energy policy.

Critics of the programme argue, in short, that if the federal government takes some forceful steps to curb energy demand in the United States, the nuclear power programme could be slowed down. The result, they argue, would be that uranium resources would not be used up so quickly, the breeder reactor would not be needed so urgently, and thousands of millions of dollars which are now being spent on the LMFBR could be channelled into the development of more acceptable sources of power such as solar energy, geothermal energy and thermonuclear fusion.

Needless to say, such arguments are rejected by energy policy-makers in the federal government. They maintain that energy demand could not be cut back sharply without precipitating severe economic problems and that, in

any case, there is no guarantee that alternative sources of energy could be developed in time to preclude the need for the breeder. To cut off LMFBR development now, they argue, could put the United States in such dire straits early in the twenty-first century that the recent Arab oil embargo would seem, by comparison, like a mild inconvenience.

The situation was summed up last June by Dr Robert Seamans Jr, Administrator of the Energy Research and Development Administration (ERDA), the agency responsible for the LMFBR programme. Seamans said that "there is no presently available or prudent alternative" to pressing ahead with research and development on the LMFBR, in the hope that technical and safety problems can be solved in time for the breeder to provide "an essentially inexhaustible source of energy to satisfy a significant share of the nation's energy needs in the next century".

The LMFBR's promise of providing a cornucopia of energy rests on the fact that, by converting uranium wastes into plutonium during its normal operating cycle, it can 'breed' more nuclear fuel than it consumes.

Present-day reactors are driven by a nuclear chain reaction which involves the splitting apart of atoms of the uranium isotope ^{235}U . But unfortunately, only about 0.7% of naturally occurring uranium is in the form of that isotope, the remainder being the isotope ^{238}U which is incapable of sustaining a chain reaction by itself. Before it can be used as a nuclear fuel, natural uranium must therefore be put through an expensive enrichment process, which essentially consists of separating out some of the ^{238}U until the ^{235}U content reaches about 3-5% of the total. At present, ^{238}U removed during enrichment is simply stored as waste, but if it is put into the core of a breeder reactor it can be converted to ^{239}Pu by absorbing fast neutrons. Since ^{239}Pu is capable of sustaining a chain reaction, it can be used as a nuclear fuel.

By the time breeder reactors begin to operate in large numbers, it is argued, enough ^{238}U will have been stockpiled at uranium enrichment plants to provide fuel for the nuclear programme for several centuries. In other words, commercial introduction of LMFBRs would do away with environmentally destructive uranium mining, and would also eliminate the costly process of uranium enrichment. Moreover, because the ^{238}U will already be available, it will not fluctuate in price.

Because of such expectations, breeder reactors came under intensive investigation as long ago as the

1940s. In the mid-1960s, the Atomic Energy Commission chose the liquid metal fast breeder (so-called because it uses liquid sodium to transfer heat from the reactor core to steam generators) as the most promising design, and a similar choice was made in Britain, France, West Germany, Japan and the Soviet Union. Then, in 1971, former President Nixon made the LMFBR programme the highest priority energy research and development programme in the United States, a position it has enjoyed ever since.

The scope and timing of the programme have been revised several times in the past few years, but the latest plan being discussed in the ERDA is geared to achieving commercial introduction of the LMFBR in the early 1990s. The chief milestones on the path to that goal will be construction in 1978 of a test reactor called the Fast Flux Test Facility (FFTF), followed by a 350-MW prototype reactor which is now expected to be completed in 1983, and construction of a large commercial-scale plant capable of generating 1,000–2,000 MW of electricity in 1987. In addition, the ERDA is supporting extensive basic research and testing efforts, and two small experimental fast breeder reactors have already been operated, with mixed success.

Virtually every part of the programme has run into problems. Schedules have slipped badly, and the cost of the effort has skyrocketed. One of the most widely publicised facts about the programme, for example, is that estimates for the total cost of research and development on the LMFBR have increased from \$3,300 million in 1969 to about \$10,700 million now. Although ERDA officials insist that the vast gulf between those estimates can be explained in terms of inflation and by the fact that earlier estimates were not rigorously derived, the spiralling costs of the programme are generating embarrassing publicity.

As for individual parts of the effort, experience so far does not generate much confidence that the latest schedules and cost estimates will be met. The FFTF, for example, was originally expected to be completed by 1973 at a total cost of about \$87.5 million, but the latest completion date is 1978 and the cost is expected to reach at least \$600 million. Designed to test breeder reactor components in a high neutron flux, the FFTF will be a fully operational LMFBR, technically capable of generating 150 MW of electricity. It is now under construction in Washington State but will not actually be used for electricity production.

Similarly, the 350-MW demonstration LMFBR, which will be the next stage in the move towards com-

mercially operating breeders, has also suffered huge cost over-runs and delays. The original completion date of 1978 has slipped to 1983, and the estimated costs have increased from \$699 million to \$1,700 million. Construction of the reactor, in fact, has not even begun; site work is now expected to begin late next year at a location on the Clinch River near Oak Ridge, Tennessee. Recent attacks on the LMFBR programme have mostly been concentrated on the budget for this demonstration reactor, and more are likely next year.

The Clinch River demonstration plant was originally expected to be followed by a series of demonstration plants leading up to a commercial breeder reactor in about 1987, but earlier this year ERDA officials revamped and streamlined the programme considerably. The idea now is to build a large testing facility for LMFBR components in the early 1980s, and to follow the Clinch River plant with a single, commercial-sized reactor in 1987. Design of that reactor will begin next year.

As recently as last March, ERDA officials told Congress that if the LMFBR programme can stay on its present schedule, more than 100 breeder reactors could be operating commercially in the United States by the year 2000. But even that estimate has since been revised. In a long range plan for energy research and development which was published by the ERDA on June 30, it was suggested that only 30–40 breeders could be anticipated by the end of the century. The breeder's impact, that report indicated, will not be felt until early in the twenty-first century.

With all those cost and scheduling problems to shoot at, the LMFBR's critics have never found themselves short of ammunition, and their assaults are expected to intensify in the next few years. The chief source of criticism at the national level is the Natural Resources Defense Council (NRDC), a public interest group whose chief activists against the breeder are Dr Tom Cochran, a nuclear physicist, Dr Arthur Tamplin, a former health physicist with the Atomic Energy Commission, and Gus Speth, a lawyer. In the past few years, the NRDC has generated reams of critical analysis of the federal government's justification for the programme, and it has received some backing on a few of its charges from the Environmental Protection Agency (EPA).

Aside from the cost of the LMFBR programme, the chief disputes will revolve around the following themes. **Uranium resources.** The ERDA has calculated that there is only enough low-cost uranium available in the

United States to provide about 30 years' worth of fuel for each of the light water reactors which will be built before the mid-1990s. In other words, domestic uranium reserves will be totally committed to those nuclear reactors which are brought on line in the next 20 years, and reactors built after about 1995 will begin running out of fuel before their useful life is finished. The ERDA therefore argues that the LMFBR's nuclear breeding capabilities will be needed to prevent the nuclear power industry from grinding to a halt.

The NRDC has challenged those assumptions on two grounds, however. It has argued that the ERDA has adopted unrealistically high estimates for the growth of energy demand, and that it has adopted unrealistically low estimates for the size of uranium reserves, both of which make commercial introduction of the breeder seem more urgent than it really is. Cochran argued before a Congressional committee last June that the commercial component of the breeder programme could be delayed for a decade without any penalty, and that the Clinch River demonstration plant should therefore be cancelled. He suggested that the delay would allow time for more accurate assessments of energy demand and uranium supply, and that the money saved could be better spent on developing solar, geothermal and thermonuclear fusion energy.

Those views have received partial support from the EPA, which suggested in a report last June that commercial introduction of the breeder could be delayed for up to 12 years without incurring economic costs. The ERDA argued, however, that work on the Clinch River demonstration plant should proceed as rapidly as possible in order to help solve some of the more pressing technical problems.

Officials from other government agencies have argued that since there is such utter confusion about long term energy forecasts, it would be foolish to slow down development of the breeder at this stage. The present policy is, therefore, to forge ahead with research and development on the LMFBR and to leave until a later date a decision about whether, or when, the reactor should be introduced commercially.

Plutonium. A large commercial LMFBR programme would inevitably entail the processing, transportation and use of huge quantities of plutonium. And that is a prospect which is worrying many people.

Tamplin and Cochran suggested in a petition to the former Atomic Energy Commission last year that if tiny inhaled particles of plutonium lodge in

the lung, they will give a massive dose of radiation to a tiny area of surrounding lung tissue. They argued that since present standards governing exposure to plutonium are based on the assumption that radiation from inhaled plutonium can be averaged over the entire lung, the permitted dose should be reduced by a factor of at least 100,000.

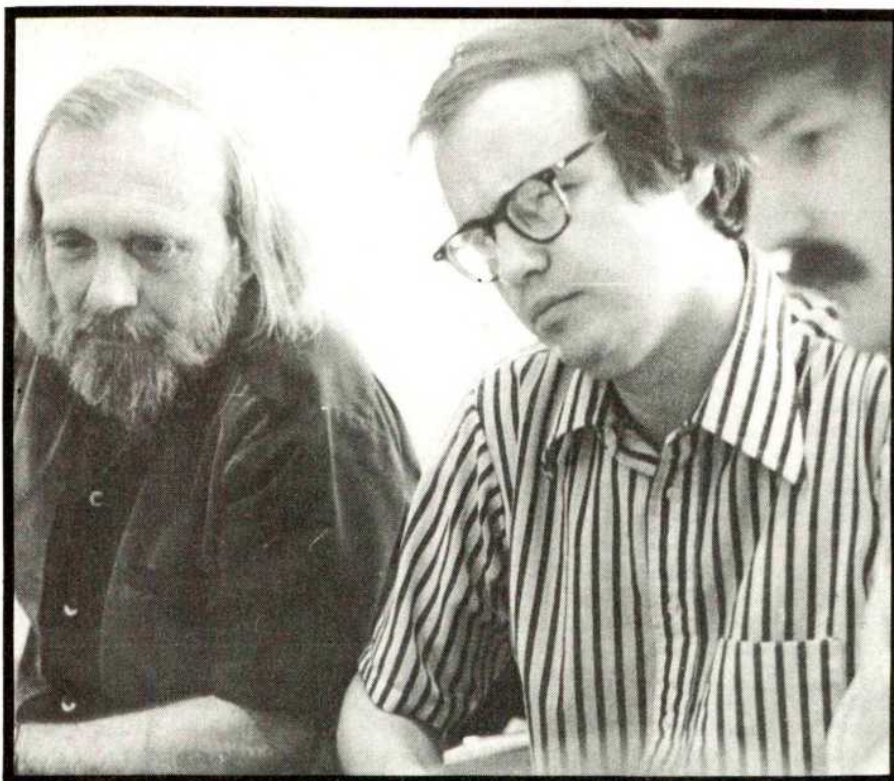
Although Tamplin and Cochran's argument has been hotly contested by scientists from the former Atomic Energy Commission and, independently, by Britain's Medical Research Council, their theory has continued to give rise to considerable discussion.

Public concern over plutonium toxicity has recently given way to even deeper concern about the possibility that plutonium could be stolen and fashioned into a crude atomic weapon. It is generally accepted that present procedures to guard the theft of potential bomb ingredients would be unsuited to a large scale plutonium industry. For that reason, the Nuclear Regulatory Commission (NRC), which is responsible for regulating the nuclear industry in the United States, recently decided that it could not yet allow plutonium to be recycled as a fuel for light water reactors. That decision will probably have to wait until 1978, the NRC says, when a number of studies of safeguard measures will have been completed.

Fears about plutonium toxicity and safeguards will obviously have to be resolved before a commercial LMFBR programme is allowed to make its debut.

Reactor safety. Although ERDA officials insist that LMFBRs would probably be even safer than light water reactors (chiefly because the liquid sodium coolant has excellent heat transfer properties and because there is a large temperature difference between the melting and boiling points of sodium), nagging doubts about the safety of breeders nevertheless remain.

And those doubts have not been assuaged by operating experience with one of the two experimental fast breeder reactors which have been constructed in the United States. The Enrico Fermi Atomic Power Plant, a 34-MW experimental reactor built in the late 1950s at Lagoona Beach, Michigan, suffered a partial core meltdown in 1966 because of a blockage in the coolant system. The accident, which resulted in radioactivity leaking from the primary containment building, was long considered the most serious mishap in the history of the nuclear power programme. Although ERDA officials rightly insist that such an accident would be extremely unlikely to occur again, the incident has nevertheless been something of a public embarrass-



Tamplin, Speth and Cochran: reams of criticism

ment to the fast breeder programme.

Officials of ERDA maintain that, in the extremely unlikely event that the nuclear core of an LMFBR should melt, the primary containment vessel which surrounds the core would prevent radioactive material from leaking into the environment; a small-scale test on a model of the FFTF has supported such an expectation. Nevertheless, a statement published by the former Atomic Energy Commission early this year acknowledged that "it must be emphasised that technical uncertainties remain to be resolved and work remains to be done before this expectation for systems other than those for which tests have been performed can be thoroughly assessed". And ERDA Administrator Seamans notes that safety questions "must be resolved satisfactorily before any decision may be made to place LMFBRs into commercial use".

Although debate over such issues has been going on in the United States for such time, Congress has only recently begun to examine the LMFBR programme in any detail. Until this year, for example, Congress has been happy to approve hundreds of millions of dollars for the programme without subjecting the matter to any discussion outside the tender confines of the Joint Committee on Atomic Energy.

But the massive cost escalation in the programme has changed all that. Earlier this year, amendments were offered to ERDA's budget authorisation bill which would have halted most of the spending on the Clinch River

demonstration plant for at least 18 months, to allow time for an independent study of the need for the LMFBR. The move failed in the House by 227 votes to 136 and in the Senate by 66 votes to 30, but critics of the programme are not disheartened. Pointing out that they have already received support from a third of the Congress after only a year's effort, they have promised to try again next year.

There is, however, only a small possibility that the LMFBR programme will be halted outright by Congressional action. For one thing, Congress would be reluctant to close down a programme which has such a large potential for meeting energy needs. And for another, the fact that several other countries are vigorously developing LMFBRs will provide a strong incentive for Congress to continue with efforts in the United States. There is also the fact that hundreds of millions of dollars have already been devoted to LMFBR development, an investment which would be difficult simply to write off.

But there is one other factor which is worth bearing in mind. If the thermonuclear fusion programme continues to make sound progress, as it has in the past few years, it will soon require massive outlays on new machines and it would then be competing directly with the LMFBR programme for funds from the ERDA's budget. The LMFBR programme has so far received priority because it is closer to commercialisation, but a significant breakthrough in the fusion programme could, conceivably, change all that. □

international news

Brazil plans 63 nuclear reactors this century

from Bruce Handler, Rio de Janeiro

BRAZIL, which gained world attention earlier this year by signing a unique nuclear technology pact with West Germany, has outlined an ambitious nuclear energy programme that will take it up to the year 2000. The president of a government-run electricity company called Furnas Centrais Elétricas SA, Luiz Claudio de Almeida Magalhaes, announced that by the end of the century, Brazil will have 63 nuclear reactors, with a total generating capacity of 81 million kW.

Magalhaes unveiled this plan in a lecture last month at the Superior War College, a strategy and policy 'think tank' for key civilians and military men which is regarded as highly important by the military-controlled government that runs Latin America's biggest nation. This speech came barely two months after the signing in Bonn of the Brazilian-German agreement, which provides for the transfer to Brazil of the know-how for enriching uranium and building nuclear power plants, along with the actual construction of eight nuclear reactors in Brazil.

Magalhaes explained that although Brazil has immense hydroelectric potential, it won't be enough to take care of the nation's voracious appetite for electricity after 1990. The country already possesses some of the biggest hydroelectric dams in the world, and it has just started building the biggest of them all, a 12-GW giant called Itaipu, across the Parana River, which should go on stream in the mid-1980s. Nonetheless, Brazil must think even further ahead.

A major problem for the development of Brazil is that its booming major cities are located either in the south-eastern part of the country or along the Atlantic coast, whereas its principal untapped rivers, in terms of hydroelectric power, are in the Amazon jungle, 2,000 miles away. And Brazilian urban areas are growing at a mind-boggling rate.

Sao Paulo, the country's industrial capital, has a city-proper population of 7 million and should overtake New York as the Western Hemisphere's largest city within a few years. The Sao Paulo metropolitan area at present has over 9 million people, and even conservative forecasts say this will increase to around 20 million by the end of the century. Rio de Janeiro has a population of nearly 5 million people, not counting the suburbs. Other

Brazilian cities such as Belo Horizonte, Recife, Porto Alegre, Salvador and Fortaleza—which most outsiders have never heard of—have already reached the 1 million mark.

Since Brazil produces only around one-fifth of the crude oil it needs every year, there is only one solution for its future energy requirements; nuclear power. Brazil's first nuclear generating station, a 627 MW installation at Angra dos Reis, 130 miles from Rio, is to begin operating in early 1978. This plant was built by Westinghouse Electric of the United States, but because of restrictions imposed by the US government, Brazil did not acquire any nuclear technology in connection with the project and it had to send uranium to the United States to be enriched. Because of Brazil's undeniable need for nuclear energy in the future, Brazilian government officials saw the nuclear security limitations dictated by Washington as extremely prejudicial and encumbering and so turned to Germany for nuclear expertise.

Two German-designed reactors of 1.3 GW each are to be built at Angra dos Reis, practically next door to the American plant, and they should come into operation in the mid-1980s. Six more German reactors, all in the 1.3-GW range, are to go on stream by 1990, serving Sao Paulo, Rio, Salvador and Recife.

Magalhaes did not say who would design and build the 54 additional reactors planned for the year 2000. Theoretically, by 1990 Brazil will have learned all the tricks of the trade from the Germans and could design the remaining reactors itself, contracting out specialised work when necessary. The Brazilian energy official said the 54 reactors will all be near big cities.

Magalhaes told his audience at the Superior War College that Brazil would be "extremely vulnerable" if it depended too heavily on hydroelectric power, although he did not elaborate on this. His main objective was to convince possible sceptics that Brazil did the right thing by making the big nuclear power push now. Magalhaes noted that Brazil's "useable" hydroelectric potential will run out by the end of the century. On paper, Brazilian rivers could provide 120 GW of power (the capacity installed at present is around 15 GW). But Brazilian officials, who have studied hydroelectrical projects in the Soviet Union, which has similar problems of distance, are less than enthusiastic about the high cost and inefficiency of long power lines.

Magalhaes was also bluntly unenthusiastic about Brazil's chances of significantly increasing oil production. The state oil company, Petrobras, which has a monopoly on drilling and refining, has been spending about \$275 million a year on prospecting, but production has remained about the same for the past several years. There are encouraging off-shore oil finds but, in spite of fantastic predictions of self sufficiency by certain government officials, it still seems too early to tell how good these new discoveries are. Meanwhile Brazil continues to spend more than US\$3,000 million a year on expensive crude oil from the Middle East. There have been suggestions that Petrobras should let big foreign oil companies look for oil in Brazil under service contracts but these have been rejected, mostly for nationalistic reasons.

At almost the same time that Magalhaes was explaining why Brazil has no choice other than nuclear energy as its prime source of energy in the future, the Mines and Energy Minister, Shigeaki Ueki, announced two new uranium discoveries. A big question mark in the Brazilian-German deal is whether Brazil actually has enough uranium to be able to carry out its ambitious plans for nuclear power. The minister said that two uranium deposits had been located in the central state of Goias and that rough preliminary estimates indicated a total of 1,500 tons of uranium ore. If this estimate holds up under further investigation, it will mean a 14% increase in Brazil's known uranium reserves. □

THE Science Research Council (SRC), hit for the second consecutive year by a reduction in its spending power, is to review its allocation of funds in an effort to maintain a balanced research programme in Britain. Several measures have been suggested, among them the provision of a larger proportion of available funds to university research, but the most interesting innovation in the long term is the introduction of industrially based research programmes designed to attract investments from commercial interests.

Speaking last week following the publication of the council's Tenth Annual Report, the Chairman of the SRC, Sir Sam Edwards, ranged freely over the council's record during the past year and its strategy for the year ahead, and made particular play of the newly approved polymer research programme established to encourage cooperation between academic and industrial interests. Spread over a period of five years, the project will operate on a budget of £2.5 million, which will include contributions from the British Plastics Federation and the British Rubber Manufacturers' Association, and will be responsible to a directorate that will "initiate and oversee a closely coordinated research programme" involving both the universities and industry.

To judge from Sir Sam's enthusiasm, the council sees the adoption of the new programme as a significant development in the running of research and plainly hopes that industrial interests will eventually be encouraged to provide support for similar programmes from which they can ultimately expect to benefit.

Certainly, the council's breakthrough in that area of research funding has come at an apposite moment. Because of inflation, the council's budget for 1974-75, just over £85.5 million, was 2% lower in real terms than in the previous year; a similar reduction is forecast for 1975-76. The effects of inflation are, moreover, aggravated by the council's commitment to the European organisations CERN and the European Space Agency (ESA), which is guaranteed in Swiss currency and which has taken up an increasing share of the budget as the pound sterling has weakened. To make the best of the remainder of the budget, which is divided between bread-and-butter support for research and facilities in the universities, and the financing of major projects which require a high expenditure in a single area of research, the council is to implement cuts in the latter sector.

Already the council has completely abandoned two of the fifteen major

projects listed in last year's report—the European X-ray satellite and the Mark VA telescope—in spite of their admitted scientific importance. And of the seven listed in the present report (which include the Northern Hemisphere Observatory and the Electron-positron Intersecting Complex—EPIC) Sir Sam declared that he could see at least two of them "going overboard" during the coming year. Questioned on which they might be, he chose not to show his hand, adding only that it

Round Britain

would cost about £74 million to adopt all seven whereas the sum available would be nearer last year's figure of £25 million. It seems reasonable to speculate, however, that with the rising cost of space research the NASA satellite project will never see the light of day.

The council's affirmation of support for university research is highlighted by the timely approval of the laser laboratory which is to be established at the Rutherford Laboratory in Chilton, Oxfordshire, at a cost of £5.7 million. It will provide a central research facility for the universities and polytechnics. The original involvement of the UK Atomic Energy Authority in the project, which was from the outset somewhat uncertain, has now been withdrawn altogether.

As far as research training is concerned, the number of postgraduates studentships will be reduced by 300 to about 3,600 in the coming year. This cut comes in spite of a 25% increase in the number of applications and means that only 14% of science and engineering graduates will be catered for. Nonetheless, the council has not been entirely negative on that front, having proposed a facelift for research training (see page 433).

More generally, the report announces the council's intention to "sharpen" the criteria used in the selection of particular fields for research funding, and though Sir Sam was characteristically phlegmatic on what precisely that means, the report itself indicates that it is the programmes considered of national importance that will receive the most favourable consideration.

● The decision to press on with the German ring accelerator PETRA in Hamburg sounds like the death-knell for its British competitor EPIC. Although the commitment of 14 million Deutschmarks is only for buildings to house the machine, approval for the machine itself cannot be far behind. Dr Godfrey Stafford, director of the

Rutherford Laboratory, at which EPIC would have been housed, was not only disappointed at the German decision, but feels it has some unfortunate implications for the future of high-energy research. The European dimension to such research, represented by CERN, and which he believes EPIC, with international funding support, would have maintained, is in danger of reverting to national rivalry. Recent unilateral initiatives by the French (with GANIL) and now the Germans cannot but put more global programmes under serious pressure.

● The United Kingdom Atomic Energy Authority (UKAEA) also presented its annual report last week, and the general tone of the peroration of its Chairman, Sir John Hill, was one of relief at the end of several years of bickering over the way British nuclear policy should proceed. During the past year the government decided to go for a steam generating heavy water reactor, while maintaining the national effort on the fast reactor; during the coming year the first two advanced gas-cooled reactor (AGR) stations at Hinkley Point and Hunterston should come on line, and the prototype fast reactor, which has been delayed by various teething troubles, should come into full operation. After 10 years of vast overspending, delays running into years, and an industrial structure which was, to say the least, unhelpful to the production of any kind of energy, the authority's chairman was wiping his brow and brightening at the prospect of a New Era in nuclear engineering. But if the progress of the £45 million Prototype Fast Reactor at Dounreay is anything to go by, the UKAEA's optimism could turn out to be the very stuff of pipe dreams.

● A piece of information from Sir John Hill which was immediately seized upon by the media was the news that Harwell had re-started a programme to examine the possibility of disposing of dangerous radioactive waste in geological strata and the ocean depths. Sir John regretted that he could not say where the Harwell men were looking or when the UKAEA might think of dumping; it was all very much a general enquiry at the moment. Quite coincidentally, the Natural Environment Research Council (NERC) announced last week that Britain was to pay \$1 million a year to join in the International Phase of Ocean Drilling (IPOD) of the Deep Sea Drilling Project (DSDP).

The term "International Phase of Ocean Drilling" is in fact a euphemism for a phase which might more accurately have been called the survival-by-a-hair's-breadth period. The DSDP, which has been supported almost unilaterally by the USA since its inception

In a controversial report published this week, a committee of the National Academy of Sciences (NAS) has convincingly demonstrated how little we know about the potential long term consequences of a full-scale nuclear war. The committee attempted to assess the magnitude of physical and biological damage in regions far removed from the target area, decades after a massive nuclear attack. Not surprisingly, it has suggested that there are huge areas of uncertainty—such as the possibility that a significant climatic change could be triggered—but it could predict no single effect serious enough to wipe out human life completely.

The report has raised considerable controversy because, aside from the emotional nature of the subject matter, it is open to a wide range of interpretations. Thus, Dr Philip Handler, President of the NAS, stated in a letter printed as an introduction to the report that the committee has concluded that *Homo sapiens* would survive the "horrendous calamity" of a massive nuclear exchange, while the Federation of American Scientists (FAS) suggested in a commentary on the report that the uncertainties in the calculations render such conclusions unwarranted.

The study, which was carried out for the Arms Control and Disarmament Agency (ACDA), took as its starting point a war resulting in the detonation of about 10,000 megatons of nuclear explosives in the Northern Hemisphere—equivalent to about half the destructive capacity of the world's nuclear arsenals. The committee concentrated its attention on phenomena likely to occur "at distances on the order of continental separations from the detonations", and it made no attempt to analyse possible economic and political consequences from such a holocaust. Findings include:

- Perhaps the most significant worldwide effect may result, not from radioactive fallout, but from massive destruction of the ozone layer. The committee notes that a major nuclear

exchange would inject huge amounts of nitric oxide into the stratosphere, which in turn could destroy between 30 and 70% of the ozone layer in the Northern Hemisphere, and about 20–40% of the layer in the Southern Hemisphere. Although much of that destruction would be repaired by natural processes in three or four

After the Third World War

by Colin Norman, Washington

years, it may take as long as 40 years for the ozone layer to be restored completely, the committee reckons.

The consequences of such an event would be a very large increase in the amount of ultraviolet radiation reaching the Earth's surface; this would damage plant life and present a severe health hazard to animals, including man. The committee notes, for example, that increased exposure to ultraviolet radiation "might have a significant impact on a great variety of [plant] species . . . and possibly could have serious implications for the ecosystem of which (particularly sensitive species) are a part". As for food crops, the committee suggests that plants such as peas and onions could be killed by large increases in ultraviolet radiation. The effect on man would be to increase the incidence of skin cancer in mid-latitudes by up to 30%, and "incapacitating cases of sunburn in the temperate zones and snow blindness in northern countries" would be expected.

- Radioactive fallout in the Northern Hemisphere would average about 1 Ci km⁻², but there would probably be "hot spots" where the fallout could be an order or magnitude greater. The committee states that "there would be no widespread effect" on plants from fallout, but in the hot spots, "minimum damage to ecosystems dominated

by radio-sensitive plants might occur". Some foods may become contaminated at levels "approaching the upper limits of present standards", however.

The effect on animals would, on the other hand, be more pronounced. An increase of 2% in the incidence of cancer would be likely, and a similar increase in the incidence of genetic disease would also result.

- As for effects on climate, the committee notes that a large nuclear exchange would inject vast amounts of dust into the atmosphere, which would reduce the amount of solar radiation reaching the Earth and possibly reduce global temperatures as a result. Moreover, destruction of a large part of the ozone layer might also lead to a reduction in temperatures. Even small changes would have serious implications for crop production, and the committee notes that "substantial changes in weather extremes . . . which could be of major importance to agriculture, have also been related plausibly to small changes in global mean climate". The committee said, moreover, that it could not rule out the possibility that a small perturbation in global temperatures "also might lead to major global climatic changes".

What are the implications of such findings for policymaking? Opinions vary. According to Dr Fred Iklé, Director of the ACDA, the committee's findings underline the futility of all-out nuclear war, because they suggest that there may be a serious ecological backlash from a massive nuclear attack.

But the FAS suggested in its statement that the conclusion that mankind might survive a nuclear holocaust has little relevance to public policy.

The committee should have recommended getting rid of all US and Soviet nuclear bombers, which would eliminate about 80% of the world's nuclear megatonnage, thereby reducing potential long-term effects of nuclear war, says FAS. □

in 1968, was expected to cost \$14 million in the present fiscal year, and the chances are that it would have collapsed in a bankrupt heap if five foreign governments hadn't agreed to bail out the project. Very broad hints were dropped during hearings of the House Appropriations Sub-committee last February that Congress might only look favourably on requests for more funds if an international interest could be demonstrated—and demonstrated in dollars and cents. The Soviet Union and Japan have also signed up for \$1 million worth of interest, West Germany is renegotiating an involvement

of two years' standing, and France is also expected to buy in at the going rate, leaving the USA to find the remaining \$9 million.

Since the information gathered by the DSDP in its pre-international phase was fairly readily available to friendly nations at something less than \$1 million a year, it seems reasonable to ask why the UK should bother laying out money. The answer is that if the UK (and the other four volunteers) hadn't, then there wouldn't have been any further information to receive at bargain rates or any other: that the UK would now have immediate access

to results, but that the emphasis in British participation was chiefly on the economic and technological benefits to be gained. This is meant to mean that the UK will be in a better position to exploit seabed resources (if and when the good ship *Glomar Challenger* happens on any) and hard scientific know-how, and that the exercise will develop the nation's deep-sea technology experience, presumably to the benefit of North Sea oil operations. Perhaps it will also provide answers to interesting questions like: "Where is there a nice spot to dump a ton of radioactive waste?" □

TO irradiate or not? That is the question. The issue involved is simple: is irradiated food—in this case, wheat—safe for human consumption? A year-old row over the matter between two premier research institutions, one of which belongs to the Department of Atomic Energy (DAE) and the other to the Ministry of Health, has recently burst into the open. And as wheat is the staple diet of much of the Indian population, the question has assumed enormous significance.

Work on the radiation preservation of foods has been going on at the DAE's Bhabha Atomic Research Centre (BARC) in Bombay for quite some time. The BARC has long been a proponent of the radiation method and has been urging the authorities to grant health clearance to specific low-dose food irradiation processes.

"Chemical disinfection methods such as fumigation require repeated application because they do not eliminate insect eggs. They may also leave harmful residues in the treated grain. Irradiation, on the other hand, is a one-shot process that completely kills or sterilises the common grain pests, their pupae, larvae and even the eggs deposited inside the grains." Commending the radiation method thus, Director of the BARC, R. Ramanna, said in a lecture at a Bangalore college last year: "The work carried out at the BARC, especially on wheat, potatoes and onions, clearly shows that preservation of foods by radiation is not only economic but safe according to all standards; the earlier we adopt this method the better it is".

But the National Institute of Nutrition (NIN) in Hyderabad has stated (in its annual report for 1974) that irradiated wheat could be hazardous to health. Specifically, it claims to have found that: (a) consumption of irradiated wheat by both animals and humans caused them to develop polyploidy—a condition characterised by more than the normal number of chromosomes in cells; (b) rats which were fed on irradiated wheat and which developed abnormal or polyploid cells transmitted these chromosomal abnormalities to their offspring; (c) mutations took place faster in animals that ate irradiated wheat; (d) there was a significant reduction in reproductive cells in malnourished rats on irradiated wheat diet; and (e) incidence of polyploidy was reduced if irradiated wheat was stored for 12 to 14 weeks before consumption.

As soon as these findings were made public the DAE responded by repudiating the NIN claim. It maintained that its own experiments (at the BARC) had not revealed any of the 'hazards' referred to by the NIN. In support, the DAE statement mentioned that, on the

basis of similar feeding trials with laboratory animals, several countries (including the USA, the USSR, Canada, France, Holland and Denmark) had declared a variety of irradiated foods as safe and wholesome for unlimited human consumption. Moreover, specialised UN agencies like the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO), it said, had rejected contentions that irradiated wheat was meant exclusively for animals and not for human beings. The DAE also refuted the charge that it had "already spent crores" in setting up laboratories

Row over irradiation of wheat

from Narender K. Sehgal, Jullundur

and equipment for radiation preservation of food.

Following this the NIN repeated its earlier warning about hazards from irradiated wheat and answered criticism of its findings. It said it had experimented with children only after "totally accepting" the DAE claim that its studies had shown irradiated wheat to be "harmless". (Although irradiated wheat was earlier found by the NIN to cause polyploidy it went ahead and fed this diet to five malnourished young children aged two to five for six weeks. This attracted severe criticism from many quarters.) But, "when we fed children irradiated wheat and found abnormal cells in circulation the study was promptly terminated", the statement added.

The DAE observation that polyploid cells in fact occurred naturally in humans and animals was countered by pointing out that in its (NIN's) experiments "feeding of irradiated wheat was consistently associated with a four- to ten-fold increase in the number of polyploid cells"; this was found to be true in rats, mice and monkeys irrespective of the protein content of the diet or the age of the animal. Although conceding that the precise significance of increased polyploidy was perhaps something that could be debated, the NIN statement pointed out that this condition had been generally associated with a kind of cancer.

The NIN denied the DAE charge that it had either underplayed or tried to conceal its own finding that the 'hazards' mentioned by it were absent when irradiated wheat was stored for over 12 weeks before consumption. But, the statement said, the NIN studies had further shown that the production of aflatoxin poison was considerably greater in stored irradiated wheat and potatoes than in the corresponding unirradiated foods. The NIN

also noted that, in the case of onions and potatoes, rotting rather than sprouting was a major problem in India and that irradiation seemed to accelerate rotting even though it did stop sprouting. (This observation was in reference to the fact that the BARC had been devoting considerable attention to preventing sprouting in onions and potatoes, instead of concentrating on the problem of rotting.)

The NIN statement said in the end that it did not look upon the matter as a prestige issue; "we are always prepared to look in all objectivity at any new evidence which may throw an entirely new light on the problem".

While the two institutions continue to exchange charges and arguments over apparently conflicting results, an expert technical committee has called for further "joint studies by the DAE and the NIN on post-irradiation storage problems of irradiated wheat and potatoes". This committee, headed by Dr M. S. Swaminathan (Director-General of the Indian Council of Agricultural Research), was set up last year at the instigation of the Prime Minister to go into the whole matter of the safety of irradiated wheat, onions and potatoes.

The committee, in its report submitted recently to the Ministry of Health, has steered clear of the safety question. In view of the problems of aflatoxin production during storage of wheat and potatoes (in the latter case, rotting as well), the government, the committee said, could consider clearing irradiated wheat and potatoes for sale only after insisting on proper storage—a minimum of six months for wheat and four months for potatoes.

The committee based its report on studies in India on wheat and on data from other countries in the case of potatoes, since none was available from India. In the case of onions, no studies had been carried out in India and the committee were of the view that studies abroad were "not yet conclusive about the safety of irradiated onions for human consumption". So it declined to make a recommendation at this stage.

In view of the serious differences between the NIN and the DAE over irradiated wheat, the Swaminathan committee called for an expert evaluation of the data by a team consisting of a statistical expert from the Maharashtra Association for Cultivation of Science and a geneticist from the Jawaharlal Nehru University. The committee also suggested that an inter-ministerial technical group should examine the techno-economic questions, the cost-benefit analyses and the feasibility of adopting radiation technology for food preservation on a large scale. □

ON August 10, on the beach at Cape Canaveral in Florida, I saw a red-headed man, sunburned to look like a boiled lobster, applying novocaine cream to his glowing back. The only unusual circumstance was that the man was Mike McElroy, whose field is the physics and chemistry of planetary atmospheres and who has loudly warned us against the ultraviolet perils of destroying the ozone layer by using aerosol spray cans. Aha, I thought: one foot in the sea, and one on the shore—to one thing constant never. For surely he, of all people, should have kept his shirt on.

The proposed ban on aerosol spray cans is a model of scientific virtue. It will save the sacred Environment of our fragile space-ship Earth from the excesses of rampant technology. It will prevent cancer. It will enable academic atmospheric physicists to cover themselves with glory—their abstruse field is Being Put To Use, while visions of research grants dance through their heads. Investigation of the subject is “a natural” for the techniques, instrumentation and vehicles of the space programme, and so it will be a first-rate spin-off for NASA. The ban may even save some metal from being used in cans, and it will benevolently force people to use their flabby muscles in wielding paint brushes. The New York Subway System will perhaps be able to clean up its cars, which are now hideously festooned with paint applied from spray cans by the vandals who also deface rocks by the roadside. Spray-on armpit deodorants will be replaced by the roll-on type, and the consumer will pay the same astronomical mark-up for a cent's worth of some aluminium salt to close the axillary sweat glands. Furthermore, no

Spray no more, ladies



THOMAS H. JUKES

one will ever be able to prove the ban was not needed—there is no neighbouring planet that could be used as a control. Private industry doesn't have a chance—the public got along very well before aerosol spray cans were introduced, and the task of the courts in denying appeals against a ban is made childishly simple by the magic word “cancer”.

Indeed the National Resources Defense Council has intoned “For every year that the banning of these aerosols is delayed, evidence shows that an additional 2,000 to 4,000 people will be stricken with skin cancer”. (Note the expletive use of the word “stricken”.) What evidence shows this, I wonder? Ultraviolet light has been reaching the skin ever since human beings first appeared. How many cases of skin cancer are caused by sunlight; what is the incidence at different latitudes; and how is it related to the percentage of the body surface that is exposed? Someone must have done some extrapolating to come up with the figure of 2,000 to 4,000 people, especially when

the effect of aerosols on the ozone layer is a matter of debate rather than measurement. And then, of course, ultraviolet light protects against rickets. . . . If the danger of cancer from solar ultraviolet light is so great, why not suggest that people keep their clothes on? From Sydney to Stockholm, the near-naked and naked white-skinned legions loll on beaches, seeking to acquire the shadowed livery of the burnished sun, and to hell with skin cancer.

A few cynical sceptics remain unconvinced. Professor Richard Scorer says it is Much Ado About Nothing. He has been rude enough to say that the talk of the threat to the ozone layer is a publicity gimmick concocted by doomsayers using scare tactics to gain headlines, and that most of the organic chlorine compounds now measured in the stratosphere are of natural origin, or from the deliberate burning of vegetation. Personally, I rather favour his thesis that the environment is full of checks and balances, and “is not as frail as some people picture it”. But heaven forbid that I should suggest that a large amount of research on this latest scare is not needed; it will train young scientists, and it will help to restore badly needed public confidence in the social values of research in the natural sciences.

And perhaps—who knows?—we may eventually be able to ride on the New York Subway, and read the advertisements for deodorants unmarred by paint from aerosol spray cans! But I am afraid that I underestimate the ingenuity of industrial chemists, who undoubtedly are hard at work to devise new spray propellants that do not destroy ozone, thus converting all our sounds of woe to hey-nony-nony.

THE first protocol of the Anglo-Soviet Joint Commission on Health Cooperation, signed on September 25, 1975 by Mrs Barbara Castle, Secretary of State for the Social Services, and Professor Boris Petrovskii, Soviet Minister of Health, represents the first stage in the implementation of the Anglo-Soviet Agreement on Cooperation in Medicine and Public Health which resulted from Mr Harold Wilson's visit to Moscow last February.

The agreement itself is a wide ranging document, dealing in general terms with “exchanges of information on new equipment, pharmaceutical products and technological developments related to medicine and public health,” reciprocal health-service arrangements for British and Soviet citizens requiring immediate medical treatment while in the other country, and the elaboration of (so far unspecified) programmes of

Healthy exchanges

from Vera Rich, London

joint research. Only one section—Article 2A—names any specific subjects for cooperation, and it is precisely these, influenza and other communicable diseases, ophthalmic diseases, and the organisation of medical care for traumatic and cardiac emergencies, which are the subject of the present Protocol.

Under the terms of the Protocol up to 25 man-months during the first year of its operation may be spent by each side on research in the other's country, with due arrangements made for the supervision of research and the pooling of results. The institutions involved in the exchange will be the

Institute of Ophthalmology of the University of London and the All-Union Institute of Ophthalmic Diseases; the Public Health Laboratory Service, Colindale, and the Moscow Institute for Virus Preparations; and the Medical Research Council Accident and Burns Unit, Birmingham, and the Moscow Institute for Emergency Medical Services.

Although the agreement makes provision for sending patients for treatment from one country to the other the Protocol stresses that in view of the “high level of development” of both health services, such exchanges are envisaged in exceptional circumstances only, and it seems likely that cases such as that of the Russian baby Irina Chudnovskaya, who has already been treated at the Brompton Hospital for a heart condition, will remain relatively rare. □

correspondence

Radon emissions

SIR,—Wendy Barnaby (August 28) writes on the problem of radon emission from the tailings of uranium milling in Sweden. This problem would arise from the large volume of uranium shale that has to be treated. She describes Professor Robert O. Pohl's report that "radon can escape more easily from the broken ground of a mine than from an undisturbed terrain". This report is well known among Swedish experts. Thorium-230, a metallic chemical element, and radon-222, a noble gas formed from uranium, can be found wherever there is uranium in the Earth's crust—not only in uranium ores and mines but also in primary rock.

In 1965, the Swedish Atomic Energy Company (AB Atomenergi) started operations at Ranstad where there is a large uranium deposit. From the very beginning, the emission of radon from the leaching residues has been studied. It has been shown that the emission from recently deposited residues is approximately equal to that measured in the open pit on the uranium shale bench. It has also been shown that the emission of radon from the restored tailings is of the same order of magnitude as that from the undisturbed terrain within the open pit area. In Ranstad, large scale tests have been made during the past few years on the deposition of leaching residues. The residues were laid out in stockpiles after mixing with fine-ground limestone and packed with a vibrating roller. After that, the area was covered with earth from the open pit and seeded.

As well as giving the landscape an attractive finish and making it possible to use the ground for agricultural purpose, the emission of radon has been minimised.

SVEN-ERIC BRUNNSJO
Department of Public Relations,
Luossavaara Kiirunavaara AB,
Stockholm, Sweden

Cancer at work

SIR,—Your article "Cancer at work" (September 18) is admirable in that it draws attention to environmental carcinogens. It is regrettable, however, that no mention is made of the painstaking work of S. A. Henry of the Medical Inspectorate of Factories in finally convincing authorities of the

risks of tar and in particular of lubricating oils used outside the cotton industries (*Cancer of the Scrotum in Relation to Occupation*, Oxford University Press, 1946). The considerable emphasis laid on the historical background leading to the recognition of these carcinogens does, moreover, distract attention from the fact that these problems are still with us. The sentence "Although less common, scrotal cancer still occurs in industry in conditions in which men are exposed to lubricating oils" is a gross understatement of the present situation. Although there are geographical variations in the occurrence of lubricating-oil cancer which are as yet unexplained, the incidence in the West Midlands not only of scrotal cancer but of skin cancer and possibly of lung cancer is a cause for considerable concern.

C. N. D. CRUICKSHANK
University of Birmingham, UK

Leprosy treatment

SIR,—Being engaged in leprosy control work in India, I daily face exactly those problems mentioned by Browne and Davey (May 15) as the main obstacles in implementing regular and sustained treatment of leprosy patients, namely, the social aspects of leprosy and the patients' attitude to leprosy.

I would like to add one more human factor and that is the importance of motivated staff. Much has been written about planning, education of the public and the approach of the leprosy patient, but little or no attention is usually paid to the field worker. Yet he is the key person in the strategy for leprosy. It is not lack of planning or of effective drugs or of a vaccine which has caused so many leprosy control programmes to founder but lack of dedicated and competent field staff. So our first concern must be to give field workers the right motivation.

My second point concerns the treatment of leprosy. Both Browne and Davey, and Crawford (March 20), refer to sulphones only but nowadays no leprosy control unit can function properly unless it has Lampréne at its disposal as well. The absolute indication for Lampréne is resistance against Dapsone. This was very rare, but according to Browne and Davey "it is not now, and the recent figures coming from countries where the problem has

been adequately investigated are very disturbing to say the least."

The greatest advantage of Lampréne, however, is that it has both bacteriostatic and anti-inflammatory properties. Thus treatment of Lampréne can be continued during reactions, whereas treatment with Dapsone is often discontinued during reactions, either by the doctor or by the patient himself. This may ultimately lead to resistance against Dapsone in lepromatous patients who are prone to reactions. It is for this momentous group of patients that Lampréne is absolutely indispensable. In Dichpalli, with approximately 10,000 leprosy patients receiving treatment, 2 to 3% require Lampréne and the proportion is still increasing.

L. M. HÖGERZEIL
Dichpalli, India

Czech conference

SIR,—I recently registered as a participant in the International Conference on Low Radioactivity Measurement and Applications (LRM) in Czechoslovakia (October 6–10,) paid for the conference fee, and got written acceptance of my participation and hotel lodgings. Also a visa for that particular conference I received from the Czechoslovakian Embassy in Bonn.

On September 17, I received a letter from the organiser (also in Czechoslovakia) to say that I would not obtain a visa to attend the LRM conference due to "news from the Czechoslovak ministry of Foreign affairs." The Bonn Czechoslovakian embassy told me on demand that the validity of my visa could by no means depend on such a letter, and that I might still travel there.

Later I received a telegram from the organiser telling me in a harsh tone and without giving reasons that "we cannot accept your participation in the conference."

I never had any political or other differences with my own or foreign countries, but I would not think of participating in this conference after such an incredible sequence of events. May I suggest to Czechoslovakian politicians: If you really want to build up effective scientific research why not treat your scientists better and their guests in a less arbitrary manner?

J. C. FREUNDLICH
Cologne, FDR

news and views

It is a long time since the discovery of bacterial transformation demonstrated that small amounts of foreign genetic information can be introduced into a living cell, and can function there. The explosive development of techniques for genetic and general biological experiments on eukaryotic cells in culture has led naturally to the search for an analogous system for the transfer of genetic information between cells of higher organisms.

Quite substantial progress has been made at several different levels, and it is now clear that the genetic information of complex organisms can be introduced into a foreign cell, and can function satisfactorily in this new environment. Whole nuclei can be introduced mechanically into the enucleated cytoplasm of another cell type (Gurdon, *Adv. Morphogenesis*, 4, 1; 1964). Somatic cell hybrids, usually made with inactivated Sendai virus (Harris, *Cell Fusion*, Oxford University Press, 1970; Ephrussi, *Hybridization of Somatic Cells*, Princeton University Press, 1972), initially also produce two or more different functional nuclei within one cytoplasm, but this is followed by nuclear fusion resulting in mononucleate cells containing chromosomes of both parental types. In many situations, particularly where the cells used are from different species, these hybrids subsequently lose chromosomes of one parental type, leading eventually to cell lines which contain relatively few 'foreign' chromosomes within their nuclei. Chromosomes introduced in this way can express their genetic information, and this system has been extensively used for the formal genetic analysis of human chromosomes (*Rotterdam Conference*, The National Foundation, 1974), studies on regulation of gene expression and so on.

An extreme example of chromosomal segregation in hybrid cells is provided by situations in which an enzymatic function of one parental type is retained in the hybrid, but no corresponding chromosome can be detected in the karyotype (Schwartz *et al.*, *Nature new Biol.*, 230, 5; 1971; Klinger and Shin, *Proc. natn. Acad. Sci. U.S.A.*, 71, 1398; 1974). The probable explanation is that a chromosomal rearrangement has occurred, translocating a small segment of the donor genome into the host cell's karyotype. Thus, a microscopically undetectable amount of foreign genetic

Information transfer in mammalian cells

from M. Bobrow and E. Solomon

information is integrated, with some degree of stability, into the host cell genome.

Many workers have attempted to demonstrate the uptake of isolated chromosomes or purified DNA by eukaryotic cells. Only recently, however, have any persuasive examples of this type of phenomenon been described. McBride and Ozer (*Proc. natn. Acad. Sci. U.S.A.*, 70, 1258; 1973) incubated isolated Chinese hamster chromosomes with mouse fibroblasts deficient in the enzyme HPRT. The absence of this enzyme makes cells unable to grow in selective medium containing aminopterin. In such a medium, McBride and Ozer isolated cell lines which had acquired the hamster HPRT enzyme, thus correcting the genetic deficiency of the mouse cells. Similar results have been obtained on incubating mouse cells with isolated human chromosomes (Burch and McBride, *Proc. natn. Acad. Sci. U.S.A.*, 72, 1797; 1975; Willecke and Ruddle, *Proc. natn. Acad. Sci. U.S.A.*, 72, 1792; 1975).

The difficulty in experiments of this sort is to distinguish genuine transfer of genetic information from mutational or induced changes occurring in the host cells themselves. Most enzyme-deficient cell lines used in selective culture systems do have a low but detectable rate of spontaneous reversion to the enzyme-producing phenotype. Results such as those of McBride and Ozer are made credible by the great care taken in characterising the HPRT enzyme in the 'corrected' cells, by a variety of techniques, as being of donor and not of host origin.

In none of these cases was there any identifiable donor chromosome present

in the karyotype of the derived cell lines. Some of these 'corrected' mouse cell lines are quite stable in culture, and are possibly again the result of small chromosomal rearrangements, with fragments of donor chromosome being inserted into the host karyotype. The size of fragment transferred must, in all these experiments, be pretty small (estimated as less than 1.5% of the human genome) as no donor enzymes have been detected in the resultant cell lines other than the one (HPRT) specifically selected for by the culture system. The reason for the integrated fragment being so small is not yet apparent. This unfortunately limits the use of the technique for genetic experiments other than those dealing with a selectable enzyme itself, or genes very closely linked to it. □



A hundred years ago

Oceanic Circulation

MR. CROLL'S statement (vol. xii, p.494), that the North Atlantic in lat. 38° is above the level of the equator, is based partly on the *Challenger* soundings and partly on Muncke's determinations of the thermal expansion of sea-water, which, however, were not made on sea-water at all, but on a saline solution prepared for him by Leopold Gmelin, according to data furnished by the incomplete analyses of Vogel and Bouillon La Grange. As Mr. Croll's statement depends on such very minute differences of volume, I am led to ask him to compare the rate of expansion of real sea-water, as determined by Prof. Hubbard, with Muncke's table; he will notice a discrepancy sufficiently wide to make it a matter of interest to ascertain how far the employment of the American observations may serve to substantiate or modify his conclusion.

G. E. THORPE

Yorkshire College of Science,
Oct. 11.

from *Nature*, 12, 514; October 14, 1875

EVERY schoolboy is told that gravity is only attractive. Unlike selenite—that amazing substance written into science fiction by H. G. Wells—ordinary matter always falls towards other matter

This asymmetric property of gravity used to pose a problem to inventors of model universes. What stops everything in the Universe from falling together at one place? During the adolescence of his theory of general relativity, Einstein applied his newly proposed gravitational field equations to a simple model universe. He was dismayed to find that this universe would not stay still—it had either to collapse or expand. Interpreting this circumstance as a defect of the theory, he set about tampering with the field equations to put the matter right. He was able to add an extra term to the equations, without compromising the basic physical principles of the theory, which did, indeed, enable a static model universe to be constructed. The result was a novel curiosity—a universe which was finite in volume, yet unbounded; a consequence of the curvature of space actually closing the universe up on itself. Included in the extra term is the 'cosmological constant'.

The physical effect of this extra term is to introduce a repulsion in the gravitational action between distant matter. This repulsion is negligible over terrestrial distances, but can dominate on a cosmic scale. By doing a balancing act between "ordinary" gravitational attraction and cosmic repulsion, Einstein's model universe could remain static.

By a quirk of fate, scarcely had the

Is the Universe running away with itself?

from P. C. W. Davies

model been constructed than it was discovered by Hubble that the Universe was not static anyway, but in a state of general expansion. New models of this expanding Universe had already been constructed by Friedmann, and the cosmological constant was not needed, which was a relief to the physicists, who naturally regarded this term as rather contrived. Since then, most cosmologists have been happy to drop the extra term (although it has been resurrected on a number of occasions, principally because it can enable the Universe to be somewhat older than its expansion rate suggests, by delaying in a "nearly static" phase). This philosophy has long fitted in nicely with observation, which has shown the cosmological expansion rate steadily slowing down with time. Such a deceleration is explained naturally by the supposition that the galaxies are pulling back on each other as they move apart. If there was cosmic repulsion, then the galaxies ought to be accelerating not decelerating. A simple calculation shows that this acceleration would actually be exponential—a cosmic runaway motion in which the Universe would for ever expand faster and faster.

During the past year, a major rethink

by astronomers has led to the suggestion that the Universe may be doing just that. This remarkable conclusion is the result of combining new observational data with theoretical calculations about a range of different astronomical studies. These include not only direct measurements of the cosmic deceleration, but information about the density of matter in the Universe and the ages of galaxies. Although all of these are subject to many uncertainties and errors, cumulative evidence has led two astronomers to claim that an accelerating universe is the most plausible model. In this issue of *Nature* (page 454), James E. Gunn (Hale Observatory) and Beatrice M. Tinsley (Lick Observatory) present their case. Although they are mindful of the somewhat shabby history of the cosmological term in general relativity ("most relativists find it repulsive on principle rather than by observation") they conclude that it is probably non-zero. Collecting together all the various constraints on the cosmological parameters leads them to suggest not only that the Universe is in runaway motion, but that it is also spatially closed, like Einstein's original model.

Naturally it will await new data and the shedding of well entrenched ideas before this model of the cosmos becomes a serious contender. If this should prove to be the case then the time will have come for the physicists to take a new look at the unwelcome appendage to Einstein's general relativity and try to understand fully the nature of the mysterious cosmic repulsion.

Sir Charles Lyell

from Martin J. S. Rudwick

The Charles Lyell Centenary Symposium sponsored by the Royal Society, the Geological Society and the British Society for the History of Science was held in London on September 1–5. It also formed the sixth symposium of the International Committee on the History of the Geological Sciences (INHIGEO).

THE name of Sir Charles Lyell (1797–1875) must be familiar to every geologist, but few perhaps have actually read his classic work on the *Principles of Geology*. Likewise historians of science often refer in passing to Lyell's work as a preparation for Darwin's, without perhaps understanding fully its importance in its own right. The Charles Lyell Centenary Symposium was designed to bring geologists and historians together for

five days of intensive discussion, not only about Lyell himself, but also about his antecedents in geological science, his contemporaries and his legacy to subsequent work.

As is usual when historians of science and working scientists meet, there was at times some tension between those who see the broader historical context as all-important, and those with greater interest in the content of the scientific ideas and observations of the period. But this mingling of professional historians with special expertise in the cultural climate of 19th century science, and geologists who often have a more detailed knowledge of particular scientific problems, was generally felt to be an enriching experience on both sides. A more interesting and more important cleavage of opinion emerged during the symposium, cutting across these lines of professional affiliation. This concerned the nature and even the reality of whatever 'revolution' or 'paradigm' Lyell may be claimed to have initiated

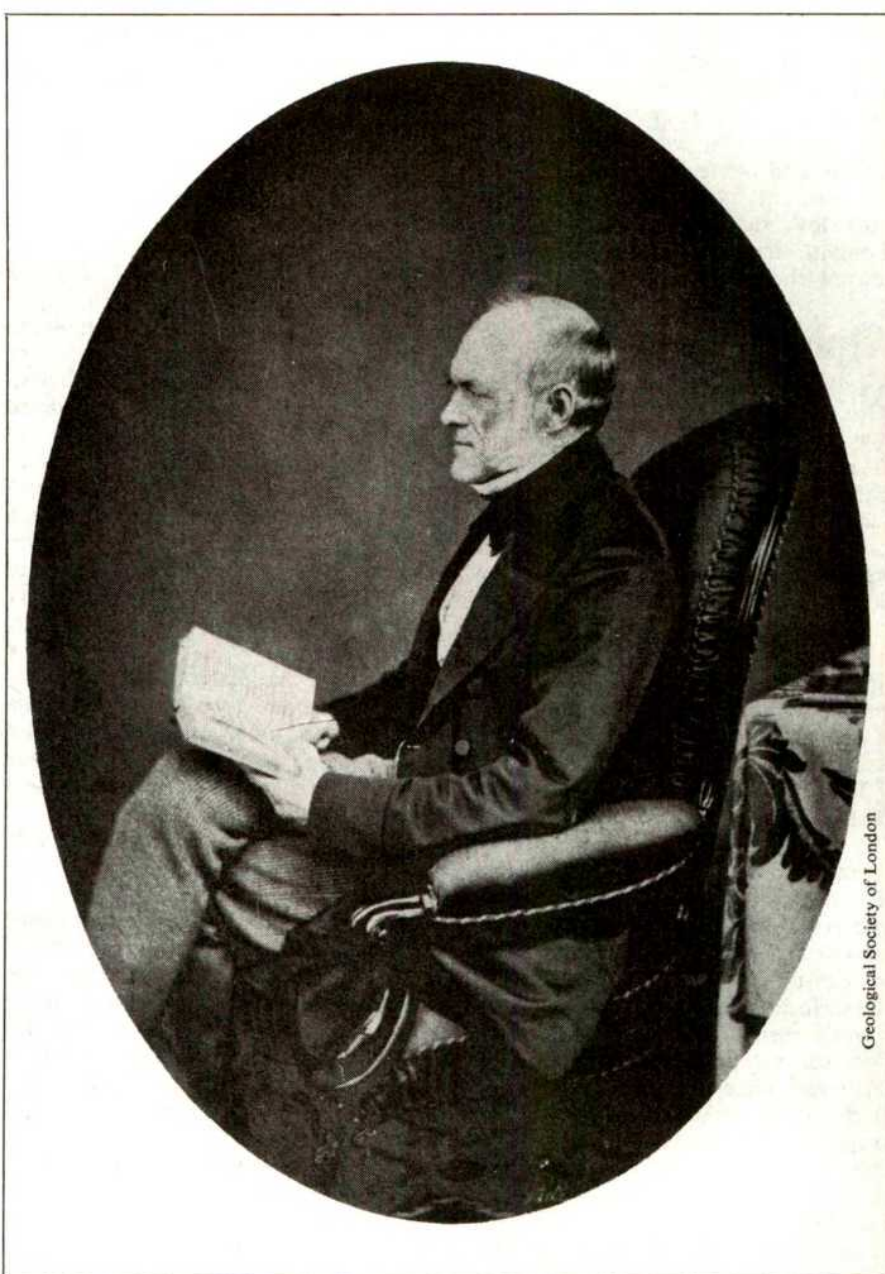
in geology. This is a topic that has been keenly debated among historians of geology in the past few years, but which has not yet penetrated much beyond the specialist literature.

After an inaugural speech by Sir Kingsley Dunham (Institute of Geological Sciences, London), the symposium was plunged at once into the heart of this controversy by a paper urging the members to apply Lyell's own scientific principles to the historical interpretation of Lyell himself! Using Lyell's own re-writing of the history of geology as his theme, R. S. Porter (Cambridge University) urged the symposium to be 'uniformitarian' about 19th century geologists, and to reject 'catastrophist' historical accounts that see Lyell's work springing miraculously out of an earlier morass of obscurantism. Several later speakers took up this challenge, enlarging on Lyell's own view of his place in the development of geology, and relating his work to that of his predecessors and contemporaries in

Britain, Sweden, Germany, Italy, France and elsewhere.

The view that there was a sharp disjunction between Lyell and his 'catastrophist' opponents was re-stated by Lyell's most recent biographer, L. G. Wilson (University of Minnesota), but without the conceptual comparison that might have led to a fuller discussion of the precise nature of Lyell's originality. Indeed, several speakers emphasised how the different meanings of 'uniformity' in geology had often been confused by Lyell himself, and had led to continuing misunderstandings in geological debate up to the present day. As C. J. Schneer (University of New Hampshire) remarked in his final summing up, what emerged from the symposium was very much a picture of Lyell 'warts and all'—a man who could be less than generous in acknowledging publicly his debts to other scientists, and whose work was perhaps less 'revolutionary' than he and his more enthusiastic admirers believed; and yet this more balanced and human picture of the man could hardly detract from our appreciation of his achievement in helping to consolidate the methods and goals of his chosen science. J. D. Burchfield (Northern Illinois University) summarised the way in which Lyell's cherished theory of a 'steady-state' earth crumbled in the late 19th century before the onslaughts of the physicists led by Lord Kelvin; but other speakers emphasised the continuing impact that Lyell's more general approach to geology had had in regions as far apart as Germany, the USA, Japan and the Pacific.

In addition to the main sessions at Imperial College, the symposium members were able to imbibe something of the atmosphere of the scientific institutions of Lyell's day, with visits to King's College and University College London, the Geological Society, the Geological Survey (as it then was), the British Museum (Natural History) and the University of Oxford, at each of which an exhibit related to the theme of the symposium was on display. In the course of these visits, a paper by J. B. Morrell (University of Bradford) showed how Lyell's own choice of affiliations to such institutions reflected their relative value to the rising professionalism of the science of that period; while M. J. S. Rudwick (Vrije Universiteit, Amsterdam) argued that visual modes of communication in published works on geology had played a decisive role in the emergence of the science. For many members, however, the high point of the symposium was the visual re-appearance of Lyell himself (ably impersonated by the symposium secretary, J. C. Thackray of the Institute



Geological Society of London

of Geological Sciences) in the lecture theatre of the Royal Institution, where a re-enactment of extracts from his early public lectures gave a vivid demonstration of his persuasive rhetoric.

Geology is a science in which the impact of first-hand experience of particular phenomena has often been deeply influential on the theoretical development of individuals. It was therefore appropriate that the symposium included two field trips, before and after the main meeting, to classic geological areas that were closely associated with Lyell: the Isle of Wight and the New Forest, where he spent his childhood and early manhood, and eastern Scotland from Berwickshire to Angus, where he was born and where he later did some of his best early research. Here perhaps the high point was the party's reception by Lady Lyell and Lord Lyell at the family home of Kinnordy House,

where books and papers from Charles Lyell's library were on display.

The symposium was highly successful in re-creating the historical context in which Lyell and his contemporaries strove to make geology a science with firmly established methods and clearly defined cognitive goals; and the widespread interest of geologists in this achievement is surely evidence of its continuing influence in the science. The superbly smooth running of the symposium, due chiefly to the chairman of the organising committee, D. A. Bassett (National Museum of Wales, Aberystwyth), might seem to make any criticism ungracious. But as R. Hooykaas (Rijksuniversiteit Utrecht and Vice-President (Europe) of INHIGEO) pointed out in his final vote of thanks, if English is to be used—as it is nowadays—as the common language of science and scholarship at international meetings, those

who have English as their native tongue must learn to be more considerate towards those who are less fortunate in this respect. As at other such conferences, too many papers by British and American speakers were delivered at ultra-high velocity and with low signal-to-noise ratio. Such thoughtlessness is hardly fair on the rest of the world. □

Alpha-clustering in nuclei

from P. E. Hodgson

THERE are now very many lines of evidence that show that the nucleons inside the nucleus tend to form clusters that persist long enough to affect many nuclear properties. The most likely form of cluster is the alpha particle because of its high symmetry and binding energy, and much evidence for the presence of alpha clusters comes from nuclear structure calculations and from measurements of the cross sections of many nuclear reactions.

The structure calculations of Brink and Castro (*Nature*, **248**, 280; 1974) showed that when nuclear matter is reduced to about one-third of its density at the centre of a heavy nucleus it tends to coalesce into alpha clusters. This suggests that in the outer regions of the nucleus, where the density is low, it is very likely that alpha clusters will be found.

In the case of light nuclei the Hartree-Fock calculations of the nuclear density show very directly the regions of maximum density, three for carbon, four for oxygen, five for neon and so on, and this suggests that these nuclei are made up of clusters of nucleons resembling alpha particles.

The familiar process of alpha decay is a very direct indication that alpha particles are to be found in nuclei. Theories have been developed that express the probability of alpha decay as the product of one factor representing the probability of an alpha particle being formed on the nuclear surface and a second factor giving the probability that it will tunnel through the potential barrier and be emitted. These theories are able to account for the very wide range of alpha decay lifetimes that occur in natural and artificial radioactive isotopes.

Many nuclear reactions also provide direct evidence of alpha clusters in nuclei. Among the most widely studied are the alpha-transfer reactions, in which an alpha particle is either removed from or added to the nucleus. Many such reactions have been studied, such as the ($^6\text{Li}, d$), ($^7\text{Li}, t$) and ($^{16}\text{O}, ^{12}\text{C}$) reactions and their inverses (*Nature*, **252**, 270; 1974). The cross sections of these reactions can be calculated by the same distorted wave theory as the one-nucleon transfer reactions by treating the transferred

particle as a single entity. On the whole this theory is very successful and allows us to calculate the probabilities of alpha formation in various states. These are the alpha-particle spectroscopic factors, which can be compared with detailed nuclear structure calculations (*Nature*, **255**, 373; 1975).

Other nuclear reactions that tell us about alpha clusters are the ($\alpha, 2\alpha$) and (p, α) reactions in which an alpha particle or a proton knocks an alpha particle out of the nucleus, but these are not so easy to treat theoretically and so have not yet given very precise information.

The (p, α) and (n, α) reactions at medium energies can give useful information about alpha clusters, since they often have a component due to the pre-equilibrium process, and if this is analysed it gives an estimate of the preformation probability of the alpha clusters (*Nature*, **245**, 12; 1973).

Reactions caused by pions and kaons have also been used to probe alpha structure, and many studies at low and high energies have provided some indications that alpha particles are present in the nucleus, and can be knocked out in favourable circumstances (*Nature*, **248**, 105; 1974; **249**, 616; 1974; **254**, 561; 1975).

It is very useful to have a clearer model of the behaviour of these alpha particles in the nucleus. Of course we know that all the phenomena mentioned can, at least in principle, be accounted for entirely in terms of the nucleons, without explicit mention of alpha particles. But nevertheless it is useful to think in terms of alpha clusters since they form an obvious physical feature of the nucleus, and if we can find a theory expressed in terms of alpha particles it will certainly be very much simpler than the corresponding description in terms of nucleons alone. We have to pay for the simplicity by some loss of precision, but this may well be acceptable if it gives us a simple model yielding physical insight into many phenomena and a way of calculating many features to fair accuracy.

Such a model can easily be constructed by analogy with the simple nucleon shell model. It is notable that all the reaction phenomena that can be understood by the nucleon shell model, in particular the optical model analysis of elastic scattering and of the resonances due to coupling to inelastic channels as well as the nucleon transfer data, are paralleled by similar phenomena for alpha particles.

This suggests that alpha-clustering phenomena can be understood by grafting on to the nucleon shell model the possibility that nucleons in outer orbits can combine to form alpha particles having a life long enough for their influence to be felt. These orbits have the quantum numbers that can easily be calculated from those of the constituent

Table 1 Theoretical and experimental alpha widths for ^{16}O

J^π	$\Gamma_\alpha^{exp}(\text{KeV})$	$\Gamma_\alpha^{th}(\text{KeV})$	$\bar{r}(\text{fm})$
	K = O ⁺ band		
4 ⁺	27	17.5	1.436
6 ⁺	125	238	1.432
8 ⁺	—	385	1.425
	K = O ⁻ band		
1 ⁻	510	675	1.536
3 ⁻	1200	1750	1.5575
5 ⁻	700	≈ 2000	1.539
7 ⁻	750	776	1.668

Data from Buck, Dover and Vary (1975).

nucleons and alpha transfers can take place to and from these alpha orbits just as nucleon transfers can take place to and from the nucleon orbits.

According to this model the alpha particles are bound by an alpha-core potential, whose eigenvalues and eigenfunctions give the centroid energies and wavefunctions of the alpha states, just as for the nucleons. It is simplest to assume that this potential has the Saxon-Woods form,

$$V(r) = V_0 / \{1 + \exp[(r-R)/a]\}$$

where R and a are radius and diffuseness parameters. Once these parameters are fixed the depth of the potential V_0 is determined by the alpha-transfer data. The only remaining parameter, the probability of alpha formation, is given by the spectroscopic factor for alpha-transfer reactions. This parameter is the only one in the model that has nothing corresponding to it in the analogous nucleon case.

Many studies of nucleon transfer reactions (*Nature*, **245**, 74; 1973) showed that the nucleon potential determined from an overall analysis of many reactions varies in a systematic way from nucleus to nucleus, and preliminary analysis of alpha-transfer reactions showed that this is also the case for the alpha potentials. This model has been applied to the alpha particle resonant states in medium weight nuclei and it is found that the energies and ordering of the eigenstates are qualitatively in accord with experiment.

A detailed study of this model as applied to light nuclei has been made by Buck, Dover and Vary (*Phys. Rev.*, **C11**, 1803; 1975). They determined the alpha potential by folding the densities of the nuclear core and the alpha particle

$$V(r) = -(2\pi\hbar^2/M)f \int \rho_A(r-r')\rho_B(r')dr'$$

where f is a real depth parameter. The densities ρ_A and ρ_B were taken from electron scattering analyses. It turns out that this potential has several advantages over the Saxon-Woods potential; in particular it can reproduce the energies of the states of low-lying rotational bands with essentially the same depth parameter.

Many studies of alpha-transfer reactions

(*Nature*, **252**, 632; 1974) have shown that they preferentially populate the rotational bands in light nuclei. This strongly indicates that these states have core + alpha structure, and it is therefore natural to interpret such states as eigenstates of the above potential.

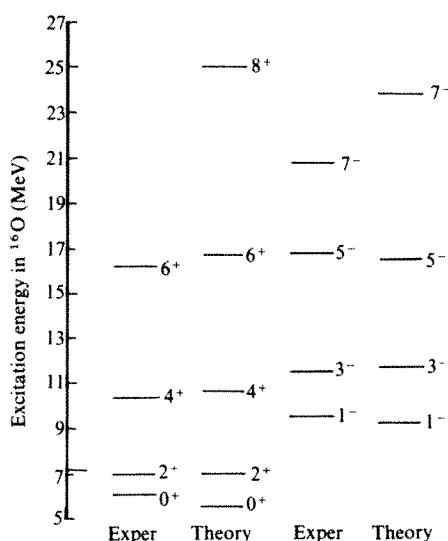
Buck *et al.* therefore applied their model to calculate the energy spectra of the $K^\pi = 0^+$ and 0^- bands in ^{16}O and ^{20}Ne , and the results for ^{16}O are shown in the figure. The parameter f was adjusted for each band, and the model then gives a good overall fit to the energies of all the states in that band. Since the widths are very strongly energy dependent, f was adjusted for each state to give the widths shown in Table 1. The required changes in f from the mean (band) values are only a few per cent. The correspondence between the measured and calculated widths indicates the qualitative reliability of the model. Since the calculated widths are very sensitive to the addition of an imaginary part to the depth parameter f , it is not correct to interpret as spectroscopic factors the ratios of experimental to calculated widths in this table.

The potentials give the alpha-cluster wavefunctions, and hence the alpha-core separations and root mean square (RMS) radii $\langle r^2 \rangle^{1/2}$ given for the ground state band of ^{20}Ne in Table 2. The values labelled $\rho^{(1)}$ and $\rho^{(2)}$ are obtained by Mosely and Fortune and by Arima and Yoshida respectively using Saxon-Woods potentials to generate the alpha-cluster wavefunctions, and $\rho^{(3)}$ is obtained from the Hartree-Fock calculations of Lee and Cusson. The model also gives values of $\langle r^2 \rangle^{1/2}$ that agree well with the empirical value of 2.9 fm given by Elton. All these models predict an anticentrifugal stretching effect. That is, the higher spin states correspond to a lower value of ρ and hence a smaller $\langle r^2 \rangle^{1/2}$. In the folding model this does not require a change in the radius of the potential itself.

As a final test of the model, Buck *et al.* calculate the electromagnetic transition rates between the states of the ground state band of ^{20}Ne , and these are compared

in Table 3 with the data and with shell model and rotational model calculations. In this calculation the effective charge was adjusted to $e_{\text{eff}} = 1.245e$ and gives values of $B(E2)$ quite close to those of the complete shell-model calculation which used $e_{\text{eff}} = 1.583$.

These results show that the alpha cluster model is able to give a good overall account of the energies and widths of the states of ^{16}O and ^{20}Ne , and the alpha-core separations, RMS radii and electromagnetic transition rates. It has considerable predictive power, since all this information can be calculated as soon as the depth parameter is fixed by comparison with the energy of a single state. It remains to be seen to what extent it will also be successful for other nuclei. It would be particularly interesting to use the alpha-cluster wavefunctions in analyses of alpha-transfer reactions, and hence obtain information on the alpha-



Comparison between the theoretical and experimental energies of the $K^\pi = 0^+$ and 0^- rotational bands in ^{16}O . The theoretical energies are calculated from an alpha- ^{12}C folded potential with depth parameter $f = 1.425$ fm for the 0^+ band and $\bar{f} = 1.55$ fm for the 0^- band. The arrow indicates the energy of the $\alpha + ^{12}\text{C}$ threshold in ^{16}O . (Buck, Dover and Vary, 1975).

Table 2 Comparison of various calculated values of the alpha-core separation and RMS radii for the ground state band of ^{20}Ne

J^π	ρ	$\rho^{(1)}$	$\rho^{(2)}$	$\rho^{(3)}$	$\langle r^2 \rangle^{1/2}$
0^+	3.67	3.82	3.75	3.86	2.96
2^+	3.68	3.85	3.78	3.84	2.97
4^+	3.58	3.81	3.76	3.76	2.95
6^+	3.44	3.78	3.76	3.67	2.92
8^+	3.06	3.48	3.56	3.54	2.85

Data from Buck, Dover and Vary (1975). All figures in fm.

Table 3 Theoretical and experimental electromagnetic transition rates for the ground state band of ^{20}Ne

Transition	$B(E2)_{\text{exp}}$	$B(E2)_{\text{th}}$	$B(E2)_{\text{SM}}$	$B(E2)_{\text{RM}}$
$2^+ \rightarrow 0^+$	57.3	57.3	52.6	57.6
$4^+ \rightarrow 2^+$	71.0	73.8	64.5	82.4
$6^+ \rightarrow 4^+$	66.0	62.7	53.4	90.8
$8^+ \rightarrow 6^+$	24.0	28.9	32.8	95.6

Data from Buck, Dover and Vary (1975). Units of e^2 fm.

spectroscopic factors and formation probabilities, which could be compared with values obtained in other ways. \square

Immunoglobulin surprises

from C. C. F. Blake

THE recent X-ray determinations of the structures of immunoglobulin Fab's and Bence-Jones proteins have enabled the crystallographers involved to correlate the structure with existing data, and at the same time to extract new and unsuspected information. Poljak in a recent review in *Nature* (**256**, 373; 1975) shows how satisfyingly the large body of chemical and immunogenetic data can be brought together in the light of the molecular structure to provide a unified view of the immunoglobulins.

In accord with this unified view Padlan and Davies (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 819; 1975) have elegantly analysed the structural variability in the combining site. They compared the structures of the V_H , V_L and C_L domains of the murine α, κ Fab McPC603 and V_L domain of the kappa-type Bence-Jones protein REI, using an interesting variation of the diagonal plot. In its more usual form the distances R_{ij} between the i th and j th atom in a molecule are plotted on a diagonal matrix against residue number and contoured at constant separation distance. Secondary structural elements produce characteristic forms of the contour surface and the whole plot reduces the three-dimensional structure to a comprehensible two-dimensional form. In the variation proposed by Padlan and Davies, appropriate for comparing similar structures, the quantities $R_{ij}^A - R_{ij}^B$ are plotted against residue numbers; where R_{ij}^A and R_{ij}^B are the corresponding interatomic distances in the two immunoglobulin domains A and B that are being compared. These difference plots show a number of interesting features, including the close structural resemblance between the V and C domains, but undoubtedly the most important is the concentration of structural differences in the hypervariable regions of the V domains. Padlan and Davies make a most telling correlation by plotting the 'average relative displacement' which represents the change in average distance between a particular residue and all the 'framework' residues in the structure from one domain to the other, against residue number. This plot bears a quite remarkable similarity to the plots of sequence variability and demonstrates very clearly that the hypervariability of sequence is paralleled by an equivalent structural variability. Thus the hyper-

variable regions, by causing insertions and deletions that change the size and shape of the combining site and amino acid substitutions that alter its chemical nature, provide the immunoglobulin molecule with an almost unlimited potential for antigen binding and antibody diversity within an otherwise constant molecular framework.

Although it is encouraging to see this model emerge from the X-ray studies, it was of course already implicit from the studies of sequence constancy, variability and hypervariability. By contrast an aspect of immunoglobulin structure that could not have been foreseen is the subject of a paper by Edmundson *et al.* (*Biochemistry*, **14**, 3953; 1975). Now that the X-ray studies have shown a structural homology between the V and C domains, it is clear that all the domains in the γ globulins have been derived by divergence from a single common ancestral gene. Edmundson and his colleagues have addressed themselves to the most important question in this context: what changes have led to the functional differentiation of the constant and variable domains? The X-ray studies have shown that the basic immunoglobulin fold has a four-stranded and a three-stranded β sheet. In the Fab's and Bence-Jones proteins the constant domains pair through the four-stranded sheets with hydrophobic interactions in a solvent-free interface, while in the variable domains the three-chain layers face each other across a solvent-filled channel that forms the combining site. On this basis Edmundson and his colleagues suggest that the V and C domains are rotational allomers and show that 38 pairs of homologous residues in the two domains are related by a rotation of 163° – 167° about an axis that passes through the intrachain disulphide bridges near the centre of each domain. They also argue that the sequence patterns in the β sheet correlate with this model. For example, in the C domain of the Mcg Bence-Jones protein the normally alternating pattern of polar and apolar residues is broken in the four-chain layers by substitution of hydrophobic residues for hydrophilic residues at the sites of interaction in the C_1 – C_2 interface. Similarly in the V domains the pattern is interrupted in the three-chain layers by substitution of aromatic for aliphatic polar residues in positions important for the maintenance of the geometry of the binding site. Edmundson believes that these genetic changes have led to rotational allomerism of the V and C domains such that homologous regions in the two domains perform different functions, and concludes that this unusual event was of critical importance in the evolution of the immunoglobulins. \square

Many models for cell growth and development

The 8th Harden Conference—Control Systems in Normal and Malignant Cells—was held at Wye, UK, on September 14–19.

from Robert Shields

THE most striking impression left by the conference is not that there are too few model systems to study growth regulation but too many. Only a few of these are easy to manipulate *in vitro* but unfortunately these are often the least interesting biologically.

Perhaps the most exciting system which is just beginning to be amenable to *in vitro* manipulation is provided by teratocarcinomas. For some time it has been claimed that teratocarcinomas contain pluripotent mouse embryo cells (termed embryonal carcinoma) but convincing evidence that this was so has so far been lacking. In an elegant demonstration R. L. Gardiner (Oxford University) showed that chimaeric mice could be obtained when the inner cell mass of mouse blastocyst was replaced by clumps of embryonal carcinoma which had been grown *in vitro*. The animals were chimaeric not only for coat colouring but also for isozyme markers, proving conclusively that embryonal carcinoma cells are truly pluripotent. The availability of large amounts of what is essentially embryonic material opens up a whole range of intriguing avenues for study using methods described by other speakers.

One of the most fascinating embryological problems is that of how position in the early embryo influences subsequent development. The great problem with this kind of study is to mark genetically an early embryo cell in such a way that its progeny can be recognised at later stages. Potentially this might be done by making metabolic mutants of embryonal carcinoma cells *in vitro* and transferring these to the blastocyst. These cells could survive in the developing embryo if they were capable of metabolic cooperation and their descendants could be recognised later in development. Work described by J. D. Pitts (University of Glasgow) suggests that metabolic cooperation takes place in many cell types *in vitro*, even between cells from species as diverse as *Xenopus* and hamster. Experiments with metabolically defective embryonal carcinoma cells might prove that similar events occur *in vivo*.

A promising *in vitro* approach to developmental processes using Friend

erythroleukaemic cells was described by P. Harrison (University of Glasgow). These cells appear to be arrested pro-erythroblasts and can be persuaded to differentiate by addition of DMSO. This provides valuable information on the timing of gene transcription and translation since globin messenger sequences can be detected using complementary DNA probes. By making mutant cells unable to differentiate and fusing these with other cells and looking for complementation it is proving possible to dissect genetically the processes that control haemoglobin production.

The sessions on cell growth control were predictably dominated by the search for the message that transmits signals received at the cell surface to the internal machinery of the cell. The current dogma is that macromolecular mitogens act at the cell membrane and do not enter the cell. As J. Kay (University of Sussex) pointed out, however, at least one macromolecule, ricin toxin, exerts its effect by entering the cell, so even the concept of surface receptors may need re-examination. A further complication is introduced by heterogeneity of mitogens and of cell types. Lymphocyte workers have often congratulated themselves on being able to work with well defined mitogens; however, the talk by F. Melchers (Institute for Immunology, Basel) showed that this is often paid for by using heterogeneous cell types in different stages of development. On the other hand, those working with cultured cells can work with a cloned cell type but have to put up with a heterogeneous mitogen, serum. In spite of this, one of the striking things about the action of different mitogens on many different cells is that the metabolic processes stimulated are very similar; what goes for the fibroblast goes for the lymphocyte. The problem is to sort out which metabolic changes cause and which are associated with cell growth.

Using pig T lymphocytes, M. J. Crumpton (MRC National Institute for Medical Research, Mill Hill) showed a close correlation between phosphatidylinositol (PI) turnover and mitogenic stimulation with a whole series of mitogenic and non-mitogenic lectins, however PI turnover was not increased by phorbol ester which is a potent mitogenic agent, showing that PI turnover is not the primary mitogenic event nor is it a necessary signal for cell proliferation. Furthermore a limited degree of lymphocyte stimulation can be produced by the calcium ionophore which also stimulates PI turnover, showing that PI turnover may be a consequence of increased calcium flux into the cell.

Another early event in the induction of cell proliferation is the stimulation of potassium entry by the Na^+/K^+

ATPase. Using cultured 3T3 cells, E. Rozengurt (Imperial Cancer Research Fund, London), could separate the serum-induced stimulation of potassium transport from the increase in phosphate transport and also from the concomitant fall in cyclic AMP levels. The role of cyclic GMP (if any) in the control of growth is still obscure. Data presented by L. Jimenez de Asua (ICRF, London) showed that different stimuli can raise cyclic GMP in 3T3 cells to the same level and yet have very different degrees of mitogenic activity. This suggests that cyclic GMP is unlikely to be the sole trigger for increased growth.

Not all relationships between the cell's interior and the environment need be conducted with chemical messages, physical interaction is also feasible, possibly through proteins which span the cell membrane. Untransformed cells display a protein of about 250,000 daltons on their surfaces, and this protein disappears when the cell is transformed. By making fluorescent antisera to this surface protein A. Vaheri (Helsinki) was able to show that this protein codistributed with actin-containing filaments which lie under the cell membrane. Furthermore, the actin filaments and surface protein disappeared together when the cell was transformed. These results suggest that the surface protein may be linked directly or indirectly to actin-containing structures in the cytoplasm. □

Waxworks for plate tectonics

from Peter J. Smith

AN oceanic ridge and the transform faults that divide and offset it are approximately orthogonal, irrespective of the overall trend of the ridge with respect to the plates on either side. The reasons for this particular geometry are still not entirely clear, or at least not beyond dispute. Most of the explanations put forward so far have involved a minimum energy criterion; in other words, it is generally assumed that the final ridge-fault configuration is that offering least resistance to plate separation. But this implies that when plates diverge the resistive forces per unit length of ridge crest are much greater than those per unit length of transform fault, an inference which is apparently inconsistent with the observation that the seismic energy released at ridge crests is no greater than that released along transform faults.

In an attempt to resolve this dilemma, Lachenbruch and Thompson (*Earth planet. Sci. Lett.*, **15**, 116; 1972)

suggested that the region in which the seismic energy is released and that chiefly responsible for the resistance to plate motion may not coincide. Specifically, they envisaged that whereas the seismic energy is released at the ridge crest in the crust which is continually breaking, the dominant resistive force is the highly viscous fluid in an intrusion zone below. But as Oldenburg and Brune (*J. geophys. Res.*, **80**, 2575; 1975) now point out, the large resistive forces invoked in this and similar models are a consequence of regarding the lithosphere as a slab of constant thickness and the intrusion zone as a narrow vertical channel beneath the ridge crest—a situation in which the plate thickness is much greater than the width of the intrusive channel. Their own model, by contrast, suggests that this condition may not obtain.

But whereas the Lachenbruch-Thompson model is conceptual, Oldenburg and Brune have constructed a real physical system. They have a tray of melted wax which is cooled until a film of solidified wax has formed between one end of the tray and a moveable paddle. When the paddle is pulled at constant velocity away from the solidified surface in which an initial zone of weakness has been produced, a miniature system of ridge segments and perpendicular transform faults is "easily attainable"—a remarkable imitation of a phenomenon discovered less than 20 years ago, using apparatus which could in principle have been constructed more than 2,000 years ago.

Whether this physical model leads to conclusions that are more or less applicable to the real Earth than those derived from its more theoretical predecessors remains to be seen. In the meantime, however, Oldenburg and Brune believe that it can provide valuable insight into oceanic ridge processes because it can be observed directly on a reasonable time scale, because quantitative measurements may be made and because the properties of the system may be varied and their effects noted. It is a simple matter, for example, to measure the thickness of the solidified surface film of wax at any point in the tray; and 'plate' thickness thus obtained is always found to increase as the square root of distance from the 'ridge'. Clearly this direct result conflicts with the constant thickness assumption built into the Lachenbruch-Thompson and other analyses.

If the wax is to be believed, the idea of high resistive forces at ridge crests may also have to be discarded, for observation shows that the ratio of the thickness of the 'plate' to the width of the intrusion zone can be quite small in the vicinity of the 'ridge crest'.

Moreover, order of magnitude calculations by Oldenburg and Brune suggest that the resistive forces per unit length of 'transform fault' are at least two, and possibly as many as four, orders of magnitude greater than those per unit length of 'spreading ridge'. Because so little is known about the physical and dynamic properties of Earth materials at depth, it is difficult to carry out comparable calculations for the real Earth. Nevertheless, assuming that the top few kilometres of the ridge crest act as a vertical channel and that the region below (defined now by a lithosphere increasing in thickness away from the ridge) is a zone of partial melt with Newtonian viscosity, Oldenburg and Brune estimate that the resistive forces along a transform fault are probably about an order of magnitude greater than the viscous forces acting beneath the few kilometres thick crust at a ridge crest of the same length.

Although such calculations must be treated with reserve, they do suggest that previous estimates of the viscous forces at ridge crests may be too high because of an inappropriate geometry assumed for the intrusion zone. Maintenance of the minimum energy criterion for orthogonal spreading would therefore require a large resistive contribution from the upper few kilometres of the ridge crest, thus restoring the seismic energy release contradiction. The only way out would seem to be to abandon the minimum energy assumption for the Earth, just as observation and calculation show it to be inapplicable to the wax model.

Instead, what seems to be crucial in producing the orthogonal pattern is the particular combination of physical properties involved. The wax model does not always lead to perpendicular ridge-fault spreading; it is necessary to use the 'right' kind of wax, and to adjust the temperature of the wax, the rate of surface cooling and the rate of spreading. The wax experiments show, however, that as far as the material is concerned the critical conditions for formation of the orthogonal pattern may be summarised as a single dimensionless parameter G , the ratio of the shear strength of the solid to the resistive stresses along the transform fault. The pattern may only be maintained as long as $G > 1$. Once this criterion is satisfied, the development of the pattern is then determined by the symmetry of the applied stress field and the ability of the wax to fracture in a brittle manner under the applied stresses. In the Earth, both symmetry and brittle fracture are assured. G is more difficult to assess, but preliminary estimates suggest that it may be as high as 30. □

Insect development

A symposium on Insect Development was held by the Royal Entomological Society in London on September 18–19.

from our *Insect Physiology Correspondent*

THE biennial symposia organised by the Royal Entomological Society of London, which have now been running for fifteen years, are unusual in that their purpose is primarily educational: to provide an opportunity for entomologists to learn in comparatively simple terms, from researchers at the fringes of knowledge, what is going on in a given field of study. The latest symposium was organised by P. A. Lawrence around the topic of the genesis of pattern development at all stages from egg to adult. Of the twelve contributors more than half came from overseas. The meeting, with an attendance not far short of 200, attracted students of growth outside the insect world and they were gratified to learn that insect development was nothing like so mosaical as they appear to have believed.

H. A. Schneiderman (University of California, Irvine) gave a general survey of the wide range of work going on in the experimental analysis of determination in the cortical cytoplasm and in the imaginal disks of *Drosophila*. He did not carry all the audience with him in his distinction between 'specification' for cell commitments which are heritable and 'determination' for commitments which are non-heritable. K. Sander (University of Freiburg) provided an introduction to insect embryology with emphasis on the nature of movements in the embryo. Evidence from exceptionally clear time-lapse photography indicates that the centrifugal movements of the nuclei at cleavage are effected by tension generated by fibrils of the nuclear asters extending to the cortex; and that the later invaginations result from tension on the blastoderm by cells in the yolk. Using a somewhat different terminology from Schneiderman, K. Kalthoff (University of Freiburg) spoke of cell determination and differentiation, but of pattern specification and realisation. Recent work by the Freiburg group has tended to support a gradient model of determination in the cortical plasma of *Drosophila*, with some evidence that masked messenger RNA may be the chemical agent. But a generalised interpretation of these observations suggested a temporal progression from a gradient state to a mosaic state, the timing varying in different insects and

being most precocious in the highly evolved forms. This sounded very much like a modernised expression of the Seidel view of things. K. Illmensee (Institute for Cancer Research, Philadelphia) had shown that genetically labelled cleavage nuclei and blastoderm nuclei from all parts of the *Drosophila* egg could contribute to any somatic structures and even to the germ line, and that the cytoplasm of the oosome could induce germ cells elsewhere.

Turning to the imaginal disks of *Drosophila*, W. Gehring (University of Basel) described the results of injection of dispersions of genetically labelled cells into wild type hosts, and their ultimate differentiation. Whereas there was already some determination at the two ends of the germ band, at the imaginal disk stage the cells were biochemically distinct. This was well seen in the aldehyde oxidase activity acquired by the antennal disk as contrasted with the wholly negative eye disk. R. Nöthiger (University of Zurich) illustrated the immense value of clonal analysis in the study of imaginal disks. Mutants induced by X-irradiation, followed by mitotic recombination, at different points in development, can demonstrate the

timing of decision taking, and the stability of the resulting determination. J. R. S. Whittle (University of Sussex) emphasised the caution needed in the use of mutants in morphogenetic studies where, for example, biochemical abnormalities could provoke pleiotropic defects in growth. P. A. Lawrence (University of Cambridge) described the important results of Garcia-Bellido and his colleagues in Madrid in the definition of 'compartments' of the integument derived from the lineage of single cells or groups of cells and visualised by X-ray-induced mitotic recombination. Even when using such mutant cells in a polyclone which far outgrows the surrounding cells, the mutant cells never intrude beyond the compartment boundary—such as exists in the longitudinal mid-line of the *Drosophila* wing or at the intersegmental boundary of the *Oncopeltus* abdomen. Recent evidence obtained in Cambridge has made clear that this remarkable restriction depends on a failure of mutual intercellular adhesion with cells outside the compartment, a property carried by special genes. The results of further studies of the significance of this new genetic unit of development will be awaited with great interest. □

Plant collections and conservation

Recommendations for the preservation of endangered plants have come from an international conference held at the Royal Botanic Gardens, Kew on September 2–6. Participants from 28 countries, meeting to consider the function of living plant collections in conservation and related research and public education, produced some resolutions calling for early action.

They include the following:

- A worldwide network of nature reserves and gardens oriented towards conservation should be established, and institutions in temperate countries should provide technical aid and personnel through the auspices of the International Union for Conservation of Nature and Natural Resources.
- Special attention should be given to the conservation of threatened floras of islands and areas with Mediterranean and similar climates, where many endemic species are endangered by human activities.
- Institutions with plant collections are recommended to give

priority to the local flora, so as to benefit from available specialist knowledge and to reduce the need for simulated climatic conditions. In this way institutions will be best able to advise and educate specialists and the public about the conservation of indigenous species.

- All governments are urged to ratify the Convention on International Trade in Endangered Species of Wild Fauna and Flora as soon as possible.
- Wherever possible, all living plant collections grown for conservation purposes should also be stored in the form of seeds.
- The propagation of rare and endangered species should be actively pursued by botanic gardens and other bodies maintaining living plant collections, and, when necessary, they should be supported by conservation or other appropriate organisations. Special attention should be given to economic plants and their wild relatives and to plants which may have commercial value.

review article

Letters from Einstein to de Sitter on the nature of the Universe

Carla Kahn & Franz Kahn*

The correspondence between Einstein and de Sitter during 1916 and 1918, recently discovered in the archives of the Sterrewacht at Leiden provides a fascinating glimpse of the thinking of the two principal progenitors of modern cosmology.

In August 1974, when the Sterrewacht in Leiden moved from the buildings which it had occupied since 1861, its archives moved with it. During the time of the removal Professor van de Hulst received a letter from Princeton. Miss Helen Dukas, Einstein's former secretary, was going through Einstein's papers, and had found some letters from de Sitter. de Sitter, who was one of the greatest directors of the Sterrewacht, was also one of the best astronomers of his time. It was thought that there might be corresponding letters from Einstein in the archives of the Sterrewacht.

In the chaos of the move from the old Observatory it was not possible to look for the Einstein correspondence immediately. It was not until we, a visiting professor of astrophysics and his wife, arrived at the Sterrewacht at about the time when the move was over, that a serious search was begun. It was suggested that one of us (C.V.K.) might index the archives, partly with a view to the possibility of finding the letters from Einstein.

Very little had been done with the papers in the past 25 years, and there was no way of finding interesting material, except by searching through all the storage boxes. In fact it was only at the thirtieth box that anything appeared which could be connected with the Einstein letters, but when they did turn up there were seventeen items of correspondence. Some were postcards and some longer letters. All but one were written by hand, and dated between 1916 and 1918. Some had been sent at intervals of about a week, and even so had obviously left time for a reply to be dispatched in between. The postal system seems to have been rather better than it is now, even though Germany was at war.

Most of the letters contained discussions of the theory of general relativity. An important purpose of Einstein's contact with de Sitter was to get this work known in England. In 1915 Einstein's first paper on the subject had appeared in Germany. But there was no communication between Germany and England at the time, and so de Sitter eventually published two long articles on relativity in the *Monthly Notices of the Royal Astronomical Society*. In preparing them he must have been stimulated to think about the subject himself and to make his own contributions. In his letters and postcards Einstein throws an interesting light on his own reactions to de Sitter's ideas.

At that time Einstein included interactions of two kinds in his theory. The first was gravitational: each mass causes a distortion of the space around itself and so affects the motion of other masses. A significant difference between Newton's laws of

gravitation and those of Einstein would only occur when very large differences of gravitational potential were involved, so that a particle moving in such regions would acquire a speed comparable to that of light. Such large potentials are likely to occur only in astronomical situations, which explains the current interest of relativists in black holes. But sixty years ago no one believed in such objects, and they are still somewhat controversial. So Einstein had to turn to the large scale properties of the Universe to find a promising application for his theory. In any case he seems to have been attracted by the age-old problem of whether space is infinite or finite, either of which seems hard to accept. To make a model of the Universe in equilibrium he needed another kind of interaction, in addition to gravitation, for with gravity alone he knew that there could be no static configurations. The general theory of relativity can be formulated so as to allow for the existence of another interaction, the cosmical repulsion. This was described in terms of the so-called Λ term, but the exact value of Λ was not predicted, and could well have been zero. (Nowadays most

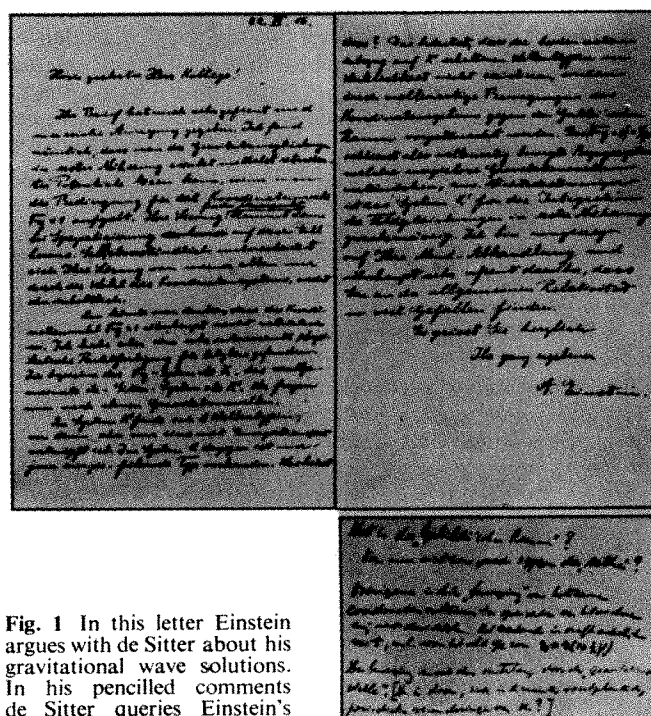


Fig. 1 In this letter Einstein argues with de Sitter about his gravitational wave solutions. In his pencilled comments de Sitter queries Einstein's opinions.

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This article was first published in Dutch in *Natuuren Techniek*, May 1975.

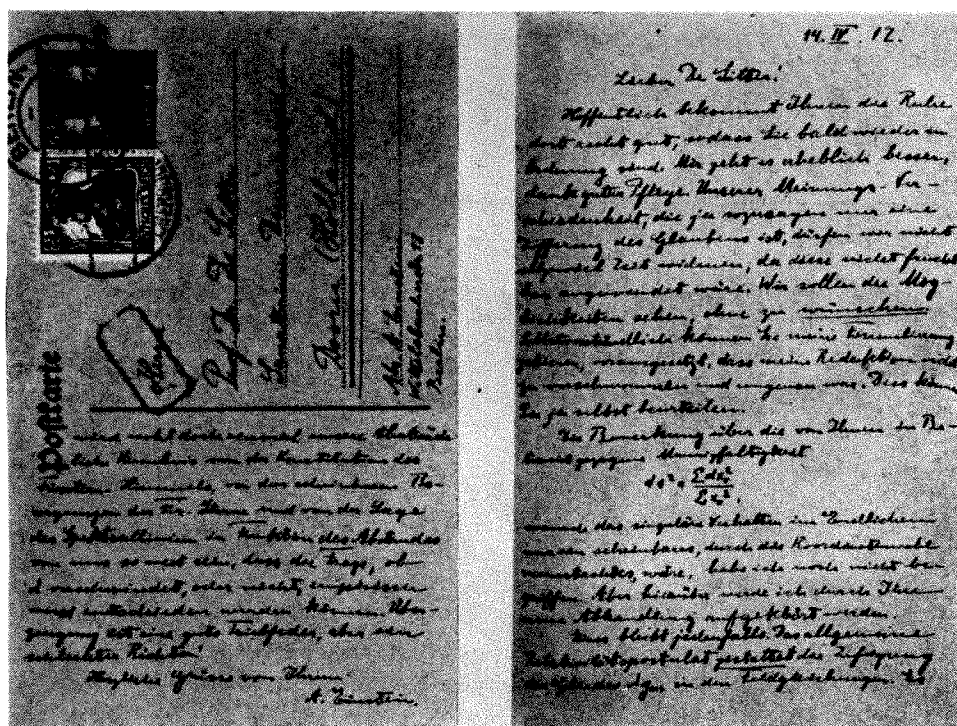


Fig. 2 Einstein expresses the view that one day our understanding of the system of stars will be good enough to determine whether Λ vanishes or not. He concludes "Conviction is a good mainspring, but a bad regulator".

relativists believe, as Einstein did eventually, that Λ is in fact zero.) But with a Λ term it was possible to balance the gravitational self attraction of the matter in the Universe, provided Λ was equal to $(4\pi/3)G\bar{\rho}$, where $\bar{\rho}$ is the mean mass density in the Universe. Naturally one has first to find out what $\bar{\rho}$ is; we shall return to this point later.

The earliest letter in the archives (dated June 22, 1916) deals with the problem of gravitational waves. de Sitter had found that there were three kinds of wave: but Einstein commented that only one kind transported energy. "What does this mean?", he asks. "This signifies that the two former types of wave . . . do not exist in reality", and he goes on to say that the waves disappear when he uses a different coordinate system, which he calls "galilean space", to express his physics.

de Sitter was not happy about this idea. In a pencilled comment he asks: "What is 'galilean space'? Can one not equally say 'the ether'?" Later he concludes that the type of motion he discovered must arise through what he called a gravitational wave.

Two more letters from Einstein continue the argument, but really take it no further. Who was right? If a physicist nowadays comes across a wave-like disturbance without energy, then he immediately suspects that the system carrying the wave is unstable. Years later Lemaître in fact constructed a model universe (with a Λ term) which begins in equilibrium but moves away from it because the equilibrium is unstable. It looks as though de Sitter saw a glimpse of this—and perhaps more—when he was thinking about gravitational waves. But he could not convince Einstein.

There then follow a letter and a card, written in November 1916 and January 1917, respectively. In the letter Einstein speculates at length on the problem of Mach's principle; does the inertia of a given mass depend on the other masses in the Universe, and if so how? This kind of question has been much discussed in the past 20 years or so, but de Sitter seems not to have been greatly interested in it. At the end of his letter Einstein says: "I do not demand in any way that you should share my curiosity". The subject does not occur again in the correspondence.

The postcard is not about scientific matters at all. "It is good of you" Einstein writes "that you are throwing a bridge over this abyss of misunderstanding. With this card you will receive the reprint you asked for, plus some others, for the colleague.

When peace returns I shall write to him . . .". The colleague was presumably Eddington and the abyss the war; Einstein sensibly does not mention him by name in case the German censor read this card.

The following post card was sent on February 2 of that year. Again there is not much science, but rather plaintively Einstein remarks: "There has been no progress with the possibility for a reappointment P., and this looks suspicious to me. There must be some intrigue at work. Naturally I shall hear nothing about it before it is too late".

He was right, for in his next letter (March 12) Einstein writes " . . . it is dreadful that they elected M. rather than K. to Potsdam, in spite of the proposal by the Academy . . . It is not clear which powers are responsible. One suspects Seliger.

"Now to our business!" The business was to construct a model for the Universe, and to tie it to some observations. Einstein's starting point was the mass density in space, as determined from star counts. He took the value $\bar{\rho} = 10^{-22} \text{ g cm}^{-3}$; from this it follows by fairly general arguments that the length scale of the Universe must be about $c/\sqrt{(4\pi G\bar{\rho})}$ if $\bar{\rho}$ is representative of all space. Einstein derives a radius $R = 10^7$ light years for the Universe, "whereas", he says, "we see to a distance of about 10^4 light years". Of course we know now that the numbers were bad estimates. The density $\bar{\rho}$ would have referred to the space in the disk of our Galaxy (and even so $10^{-22} \text{ g cm}^{-3}$ is about thirty times larger than the value astronomers have determined now). The density averaged over the Universe is many orders of magnitude smaller, and our present value for the radius of the Universe consequently much larger. Finally the distance "to which we see", at least in the plane of the Galaxy, is restricted by the obscuration due to interstellar dust, and has little to do with the Universe at large.

Einstein was uneasy about the results of his investigation. "I compare space to a cloth . . . one can observe a certain portion . . . we speculate how to extrapolate the cloth, what holds its tangential tension in equilibrium . . . whether it is infinitely extended, or finite and closed. Heine has given an answer in a poem, 'and an idiot expects an answer'". And then he looks forward to his next meeting with de Sitter in Leiden. Now, almost 60 years later, we astronomical "idiots" are still waiting for the answer.

A few days later (March 24) Einstein wrote again. de Sitter had developed a model for the Universe, but Einstein did not

times one can put a rigid circular hoop into your Universe which has no place in it at time $t = 0$ ". The 'Big Bang' evidently did not appeal to him.

But he accepts it in a letter one week later (June 22) in which he gives a geometrical argument to explain why the instant of the Big Bang is special. "This point is thus *de facto* preferred . . . Naturally this does not constitute a disproof, but the circumstance irritates me".

There are several further communications from Einstein after this letter, but they are concerned more with details than with principles.

There are also less scientific parts of the letters. de Sitter and Einstein seem to vie with each other in describing their ill-health; in fact most of the letters are addressed to de Sitter at a sanatorium. Together with this correspondence there were some letters from Eddington, who was Secretary of the Royal Astronomical Society at the time. They concern the papers which de Sitter had written on general relativity. The RAS eventually published them, but at first Eddington's letters were somewhat cool. You understand, he wrote, that every paper published by the RAS has to be sent to a referee. (He would of course have been quite qualified to referee them himself.) We are having trouble with the printers, the paper is longer than we normally print and so on. But in the end the RAS accepted de Sitter's contribution.

Another story which is intriguing has to do with the removal of the Radcliffe Observatory from Oxford to South Africa. It had been set up by John Radcliffe in the eighteenth century, and hardly any student had used it for the past hundred years. (It was Dr Knox Shaw, the Radcliffe observer, who originally proposed that his observatory be moved away from Oxford. In one of his letters to de Sitter he remarks that the students

had made practically no use of his observatory for a very long time. Yet one of Einstein's chief arguments against the removal was that one could not justify the loss of amenities, which the University of Oxford would suffer as a consequence.) Oxford is not blessed with the kind of weather that facilitates observing. A number of influential astronomers felt that it would be useful to move the observatory to Johannesburg, but there was some opposition, led by Lindemann, who was at that time an influential scientist in Oxford. He had enlisted the support of Einstein, while de Sitter was a supporter of the astronomers. Because the observatory was administered by a Trust Fund, the matter had to be decided in the High Court.

de Sitter wrote to Einstein to say that he had seen a letter over Einstein's signature, and that he [de Sitter] could not believe that Einstein knew what he was signing. Einstein wrote back rather crossly that of course he knew what he was signing. de Sitter then sent an affidavit to the proposers of the move in which he said that, though Einstein was a very great scientist, his knowledge of astronomy was minimal and his advice in this matter should be ignored. Counsel for the proposers felt that such a letter would not be helpful to their cause, and de Sitter was asked to remove the reference to Einstein from his affidavit.

The last letter from Einstein was written in 1933. He thanked de Sitter for his offer of help, but said that he was managing "to survive with his own (family)", and was even able to help others "over the water". He did not expect to be able to save very much from Germany, as proceedings had been started against him for high treason.

Soon after this Einstein moved to Princeton, where he lived and worked until his death in 1955. de Sitter's health failed, and he died in 1934.

articles

An accelerating Universe

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New data on the Hubble diagram, combined with constraints on the density of the Universe and the ages of galaxies, suggest that the most plausible cosmological models have a positive cosmological constant, are closed, too dense to make deuterium in the big bang, and will expand for ever. Possible errors in the supporting arguments are discussed.

"If then, Socrates, in many respects concerning many things—the gods and the generation of the Universe—we prove unable to render an account at all points entirely consistent with itself and exact, you must not be surprised. If we can furnish accounts no less likely than any other, we must be content."

Plato, *Timaeus*

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THE cosmological constant has been invoked on several occasions to correct some seemingly real difficulty with the cosmological predictions of standard general relativity^{1,2}. The most notable of these were its initial use by Einstein to produce static universes, the models by Lemaitre designed to explain how the Solar System could be older than the then accepted value of the Hubble time, and most recently by Petrosian *et al.*³ in an attempt to explain the concentration in QSO number counts near $z = 2.0$. In each of these cases either the rationalisation for the introduction of non-zero Λ has disappeared with better data or understanding, or the application has been unsuccessful in its intended purpose.

We seem to be in the situation once again where the data may call for Λ to be dusted off and inserted in the field equations. The rationale now is the almost-zero formal value for the deceleration parameter obtained by Gunn and Oke⁴ which, when reduced by any reasonable evolutionary correction, yields negative values for q_0 much larger than the formal errors.

Such negative values correspond to accelerated expansion and require a net repulsive force. Within the framework of general relativity, a positive Λ is the only way to obtain such a force. (It is perhaps worth noting that relativistic theories of gravity exist which are consistent with Solar System experiments which do not satisfy Birkhoff's theorem and in which accelerated cosmological models arise naturally (C. Caves, unpublished).)

The physical situation regarding Λ is unclear. Most relativists find it repulsive in principle rather than by observation. If it is regarded as a fundamental constant of the classical theory, it does indeed reduce the lustre of an otherwise beautiful framework. Zel'dovich⁵, however, has shown that a Lorentz-invariant stress tensor may arise quite naturally out of quantum fluctuations *in vacuo*, and if so, its form must be $\Lambda g_{\mu\nu}$. If the cosmological term is to be regarded as a piece of the stress tensor rather than a part of the theory, it would seem to be more acceptable.

In any case, comparison of the classical Hubble diagram with density estimates based on 'local' determinations seems the best way of determining the existence of a non-zero Λ . We shall see, in fact, that the Hubble diagram alone may dictate that Λ be non-zero.

The data do not demand this conclusion, and there is the possibility that systematic errors remain, but the suggestion is strong enough that we thought it worthwhile to investigate the models in the light of other relevant observations, in the spirit of the recent work of Gott *et al.*⁶ for models with $\Lambda = 0$.

Properties of Friedman models with $\Lambda \neq 0$

Three parameters specify a model completely. A set usefully related to observables is the density parameter

$$\Omega = 8\pi G\rho_0/3H_0^2$$

(where G is the gravitational constant, ρ_0 is the present density and H_0 is the Hubble constant), the deceleration parameter q_0 ($= -\ddot{R}_0 R_0/\dot{R}_0^2$, where R_0 is the present expansion scale factor), and H_0 . An alternative set that is related to the nature of the function $R(t)$ is the ratio Λ/Λ_c (where Λ_c is the critical value, $\Lambda_c^{-1/2} = 4\pi G\rho R^3/c^2$), which remains constant during evolution of the Universe—and a dimensionless scale factor to specify the present epoch, such as $kR^2\Lambda_c$ and a scale such as H_0 . (Relationships among these quantities are given in refs 3 and 7.) We note now that $\Lambda/3H_0^2 = (\Omega/2) - q_0$, so if $q_0 < 0$, Λ must be > 0 .

Figure 1 depicts the model types in the $(q_0, \log \Omega)$ plane, with small inserts showing schematically the function $R(t)$. Lemaître models, with an extended quasistatic period, occur for Λ only slightly greater than Λ_c . Note that space is hyperbolic, flat, or spherical according to whether the quantity $(3\Omega/2) - q_0 - 1$ is negative, zero, or positive: but if $\Lambda > 0$, closure no longer means necessarily that the Universe will stop expanding, whereas for all models with $q_0 < 0$, the expansion continues indefinitely.

Constraints

To establish constraints on possible model parameters, we need a third dimension (H_0) in Fig. 1. In Fig. 2 this is represented partially by separate $(q_0, \log \Omega)$ diagrams for two values of H_0 , using an expanded scale in the area that will include any viable models (the parameter space studied by Gott *et al.* is represented by the lines $\Lambda = 0$).

Maximum redshift. If $q_0 < -1$, then models with a maximum redshift, z_m (that is a minimum R) occur if $\Omega < \Omega_c(q_0)$, the value of Ω on the line $\Lambda = \Lambda_c$. The value of z_m at a given q_0 is then less than a critical value $z_c(q_0)$, shown in several places in Fig. 1. The existence of a QSO at $z = 3.53$ immediately shows that if $q_0 < -1.13$, models below the critical line are ruled out. More seriously, thermalisation of the background radiation requires a redshift of at least 100. Models with such a

large maximum redshift have $\Omega < 2 \times 10^{-6}$, which is impossible, so we can eliminate at once the whole parameter range $q_0 < -1$, $\Lambda < \Lambda_c$. This constraint is not shown in Fig. 2, since it will be implied by a more stringent limit later.

Ages of galaxies. A lower limit to the age of the Universe (t_0) is given by limits for the ages of stars and elements in the Galaxy, $\approx 8 \times 10^9$ yr (ref. 6). This rules out models with $H_0 t_0 < 0.40$ and 0.64 for $H_0 = 50$ and $80 \text{ km s}^{-1} \text{ Mpc}^{-1}$ respectively, which lie above and to the right of the areas shown in Fig. 2 (compare the $H_0 t_0$ contours in Fig. 1), so will be eliminated by stronger constraints.

To set an upper limit is more complicated. A reasonable upper limit to the ages of globular clusters is 16×10^9 yr (compare the references cited in ref. 6); nucleochronometers cannot provide a model-independent upper age limit for the Galaxy⁸.

Evolutionary models for giant elliptical galaxies predict that their colours would be redder than is observed for ages greater than 16×10^9 yr, if their composition is solar (B. M. Tinsley and J. E. Gunn, see ref. 20). Their spectra indicate an overabundance of metals, if anything, relative to the Sun⁹⁻¹³, so we take as a safe upper limit 18×10^9 yr, corresponding to an underabundance by a factor of 2. Though it seems natural in most theoretical scenarios to form galaxies at a time roughly equal to the dynamical time of the galaxy (a few times 10^8 yr), galaxies can form later, given enough dissipation to enable them to evolve to their present condensed states. The perturbation equations (see, for example, ref. 14) require that galaxy formation occurs before the time when Ω becomes small. This is a significant constraint for small H_0 ; the locus of those models for which $\Omega = \frac{1}{2}$ 18×10^9 yr ago is shown in Fig. 2a. Models near this limit in the $k = +1$ region have relatively small redshifts at the latest allowable epoch of galaxy formation and are thus afflicted with very large evolutionary corrections (see below) and are inconsistent with the limits on the blue-visual cosmic light^{15,16} unless the radiation from

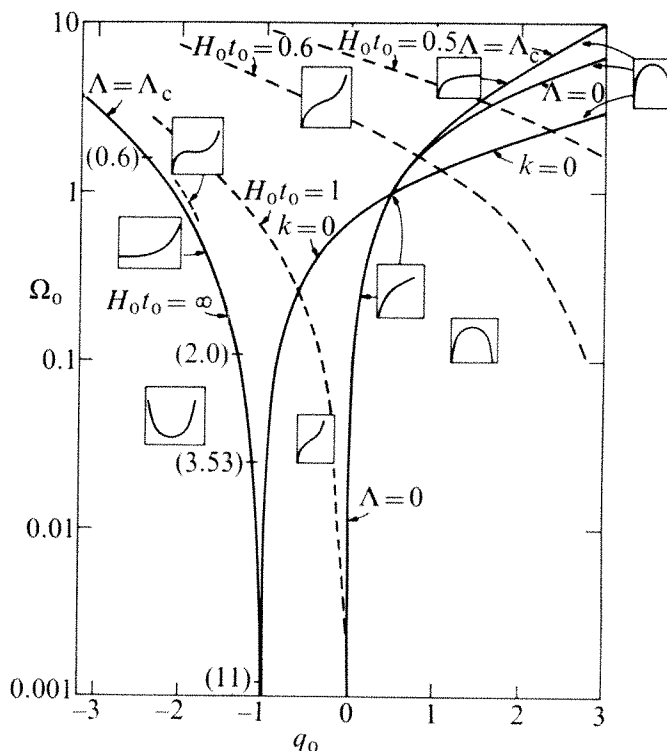


Fig. 1 Friedman models in the $(q_0, \log \Omega)$ plane. Solid curves show the models with $\Lambda = 0$, $\Lambda = \Lambda_c$, $k = 0$ as indicated. Dashed lines give dimensionless ages, $H_0 t_0$ (which are infinite at and below the critical curve for $q_0 < -1$). Small inserts show the expansion function $R(t)$ schematically. Numbers in brackets on the critical curve show the limiting redshift for asymptotic (Eddington) models on this curve, which is greater than the maximum redshift in models below or to the left of that point.

young galaxies is strongly absorbed. Galaxies can form shortly after decoupling at large redshifts only if $t_0 \lesssim 18 \times 10^9$ yr; this line is shown in Fig. 2a and b. For $H_0 = 30 \text{ km s}^{-1}$, all models with $\Lambda \geq 0$, $q_0 < 1$ are older than this.

Density. A lower limit to Ω is the contribution, Ω^* , of matter associated with galaxies. Gott *et al.* estimate $0.04 \lesssim \Omega^* \lesssim 0.08$, and we consider that an interval no greater than $0.02 \lesssim \Omega^* \lesssim 0.3$ is consistent with all the evidence discussed. The dynamical effects of Λ will invalidate an estimate of Ω^* in a region with $\rho < |\Lambda|/4\pi G$, that is, with density contrast $\rho/\rho_0 < 2|\Lambda|/$

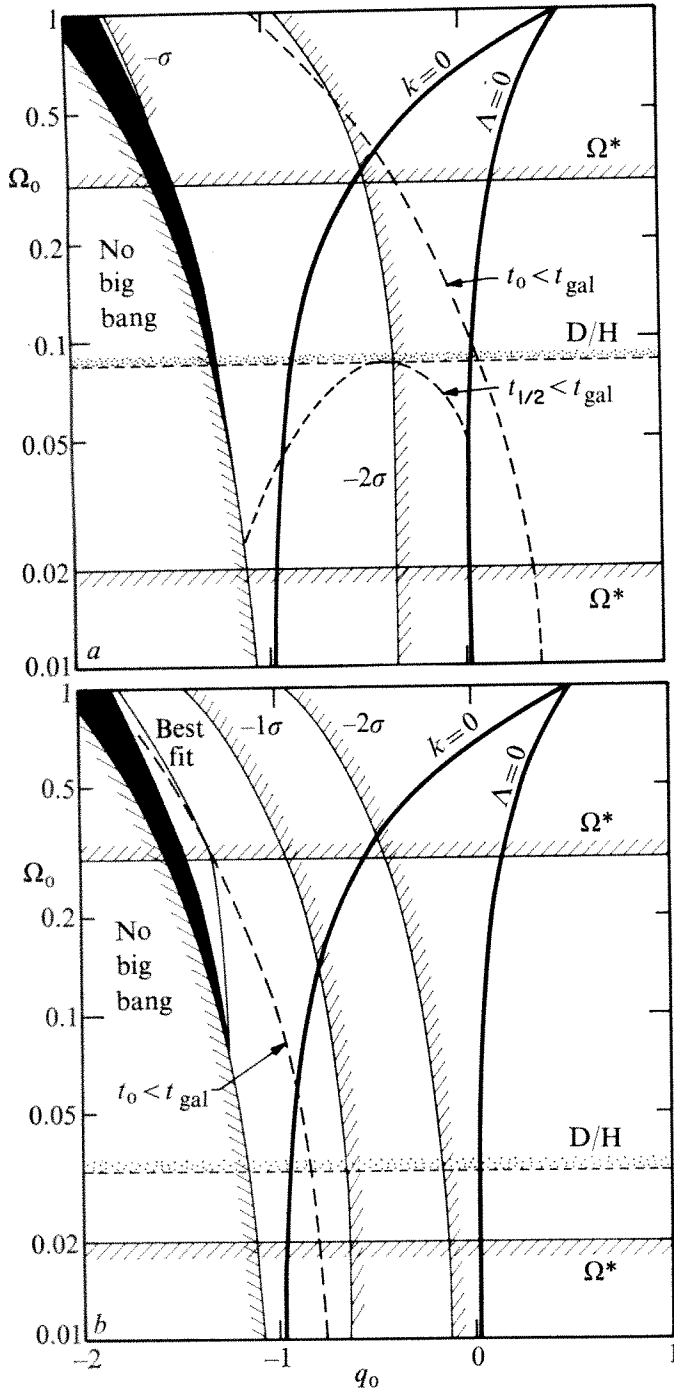


Fig. 2 Constraints in the $(q_0, \log \Omega)$ diagram for: a, $H_0 = 50$ and b, $H_0 = 80 \text{ km s}^{-1} \text{ Mpc}^{-1}$. Heavy lines correspond to $\Lambda = 0$, $k = 0$ as in Fig. 1. The two boundaries labelled Ω^* are limits on the dynamically detectable density, and the density limit D/H is an upper bound if deuterium is cosmological. The solid black area is for models with $0 < (\Lambda/\Lambda_c) - 1 \ll 1$ which are ruled out by the absence of focusing effects; all models with $\Lambda < \Lambda_c$ (no big bang) are ruled out. Constraints based on the ages of galaxies (t_{gal}) are discussed in the text, as are those based on the Hubble diagram and labelled best fit, -1σ , and -2σ .

$3\Omega H_0^2 = |\Omega - 2q_0|/\Omega$. All of the models that will be allowed in Fig. 2 have $(|\Omega - 2q_0|)/\Omega \lesssim 100$ so they are consistent *a posteriori* with the use of Ω^* from the dynamics of bound clusters. It may not, however, be valid to derive an upper bound on Ω^* from the uniformity of the Hubble flow in some models.

Figure 2 shows the lower bound $\Omega > \Omega^* > 0.02$, which is independent of H_0 .

An upper limit to Ω is not so safe because matter could be hiding outside galaxies or clusters⁶. The requirements on smoothness are less severe if $|\Lambda|/4\pi G$ is significant compared with the mean density, but the least contrived situation is that $\Omega \sim \Omega^* < 0.3$. This value is shown as an upper bound in Fig. 2.

A more stringent upper limit is obtained if we believe that deuterium is produced in the big bang. Studies of nucleosynthesis in supernova shocks, subsequent to those cited by Gott *et al.*, have not provided a clearcut answer as to whether supernovae could be the source of galactic deuterium^{17,18}.

If production in a 'canonical' big bang is required, then the best estimate⁶ is $\rho_0 < 4 \times 10^{-31} \text{ g cm}^{-3}$, which is indicated as a possible upper limit to Ω in Fig. 2.

Quasars. In Lemaitre models, a peak in the redshift distribution of extragalactic objects is predicted at the inflection redshift z_i , at which q changes sign from positive to negative; its value is given by $(1+z_i)^3 = (\Omega - 2q_0)/\Omega$. From the absence of a significant peak in QSO counts at $z < 2$, and from the calculations of Petrosian and coworkers^{1,3,19} we believe that models with $1 < \Lambda/\Lambda_c < 1.1$ ($k = +1$) and $z_i < 2$ can be ruled out. In fact, we believe that all ($k = +1$) models for which the radial coordinate reaches π at a redshift $z\pi < 3$ can be ruled out. These models have a range of redshift around $z\pi$ for which objects are very bright, and it is unlikely that bright galaxies and quasars from such redshift ranges would have escaped detection. This limit is a bit more stringent than the one applied to the counts, and the forbidden region is indicated by the solid black area at the left of Fig. 2a and b.

Hubble diagram. The interpretation of the Hubble diagram is quite complex, and its understanding is contingent on understanding the evolution of elliptical galaxies. Ignoring evolution, Gunn and Oke⁴ get an apparent value $q_{0a} = -0.15 \pm 0.57$ (1σ) for their favoured statistical sample, from a set of galaxies most of whose weight for this problem comes from redshifts in the vicinity of 0.3.

This is consistent with the classical steady-state value $q_0 = -1$, within the errors, in which case no evolutionary correction can be applied. The steady-state model seems, however, to be very unlikely on other grounds, such as production of the microwave background radiation.

The evolutionary correction from population syntheses of ellipticals is (ref. 4 and B.M.T. and J.E.G., unpublished).

$$\Delta m(z) \simeq -(1.3 - 0.3x) \ln \left\{ \frac{t_0 - t(z)}{t_{\text{gal}}} \right\}$$

where t_0 is the present age of the Universe, $t(z)$ is the age at z and t_{gal} is the present age of the Galaxy; x is the slope of the initial mass function. The first order correction to q_0 from this magnitude change is

$$EC = q_{0a} - q_0 = \frac{2.5}{\ln 10} \frac{2}{2-\alpha} \frac{1.3-0.3x}{H_0 t_{\text{gal}}}$$

where $\alpha \sim 0.7$ is the slope of the flux-diameter relationship for ellipticals⁴. For reasonable values of the parameters, EC is always positive.

Results on red and infrared line indices demand that $x \leq 1$ if the initial mass function can be represented by a power law (B. M. Tinsley and J. E. Gunn, unpublished). We have thus taken $x = 1$, since this must underestimate $|EC|$, and we have

likewise taken the largest possible number for t_{gal} ; that is, t_0 or 18×10^9 yr, whichever is smaller. We then constructed Hubble diagrams for a set of models in the Ω, q_0 region of interest ($\Lambda \geq 0$, $0.01 < \Omega < 1$, $\Lambda > \Lambda_{\text{crit}}$ for $k = +1$). The Malmquist correction was applied as outlined previously⁴ for a dispersion in absolute magnitude $\sigma = 0.4$. The standard deviation in q_{0a} was then converted into a magnitude difference at $z = 0.3$. This simplification enables the goodness of fit of any model to be checked approximately by calculating its predicted magnitude at $z = 0.3$ and comparing this with the value given by q_{0a} with no EC and the 1σ and 2σ limits given by the formal errors on q_{0a} . The approximation should be very good, since the relevant data are strongly concentrated at about this redshift, and the comparison is, to first order, independent of redshift. The results, in the form of best fit lines and contours at 1σ and 2σ are plotted on Fig. 2a and b. All models are ruled out at the 2σ level if H_0 is as small as 30.

For $H_0 = 50$ (Fig. 2a), there is almost no model which agrees within 1σ , none which satisfies the Ω^* constraint, and certainly none which makes deuterium. At the 2σ level, a wide variety of models is possible, including models of both curvatures. None of these models which satisfy the Ω^* constraint can make galaxies early; and all must have positive Λ .

For $H_0 = 80$, the ages are much smaller, and the age constraint on the galaxies easier to satisfy. A larger collection of models can make galaxies early, and thus the evolutionary corrections can be smaller. There is a small portion of the best fit line in the allowable region on this diagram, but none of these models can make deuterium, and all are close enough to $\Lambda = \Lambda_{\text{crit}}$ that they have peculiar Hubble diagrams at large redshifts ($z \sim 2-3$), and may be ruled out on that basis in the future. All are closed. Models at the -1σ and -2σ levels can make galaxies early, can make deuterium, and can be either open or closed. Again all require that $\Lambda > 0$, but the -2σ contour approaches the $\Lambda = 0$ locus very closely for small Ω . It is perhaps worth noting that if the Ω^* level is put in at the 'best' value of 0.06 found by Gott *et al.*, no models with H_0 this large can make deuterium. All of these remarks about deuterium of course refer to the 'standard big bang' nucleosynthesis models and can be relaxed somewhat if one considers various complications (see ref. 6).

Summary of constraints. Thus it would seem that the data suggest (1) that the universe is closed, (2) that the density is fairly high ($\Omega \gtrsim 0.1$), (3) that $\Lambda > 0$, and in fact, that Λ is within a factor 2 of Λ_{crit} , (4) that H_0 is at least about $80 \text{ km s}^{-1} \text{ Mpc}^{-1}$, and (5) that deuterium is not primordial. We are, of course, not compelled to believe all of these conclusions, since within 2σ of the best fit are a large number of other possibilities, but no models with $\Lambda = 0$; this state of affairs is independent of H_0 , but can become more stringent if H_0 is small enough that galaxies are made late. All of the even remotely allowable models expand forever. The $q_0 = 1/2$, $\Omega_0 = 1$ model which is the 'closest' boundary point of the set of $\Lambda = 0$ models in the $q-\Omega$ diagram in which the models undergo collapse in the future is 4σ away from the observations.

Conclusion

The first reaction to all this is that something must be terribly wrong, and we conclude therefore with some of the possible pitfalls.

Systematic effects in the photometry. Since the low and high redshift samples in Gunn and Oke's analysis⁴ had to be treated differently due to technical difficulties, there is a possibility that the two sets are not corrected properly to the same system. It seems highly unlikely that the error is as large as 0.05 mag, however, which corresponds to an error in q_0 of less than 0.25. An increase in the measured q_0 by this amount would make the fitting problem less extreme but would not substantially change the situation presented here.

Intergalactic obscuration. An absorption of 0.2 mag at $z = 0.3$ will decrease the measured q_0 by 1. This requires a gas density

corresponding to $\Omega \sim 0.03$ if there is the same dust-gas ratio as the interstellar medium in the solar neighbourhood. The presence of so much dust seems *a priori* exceedingly unlikely, but it may be possible to create scenarios in which this much nuclear processing occurs and is followed by ejection of grains into intergalactic space.

Galactic evolution. The evolutionary corrections are of course crucial to the interpretation given here, and one may reasonably ask how uncertain they are. In our opinion there are two major sources of uncertainty. First, the power-law approximation to the main-sequence mass function may be seriously misleading. Until careful and accurate evolutionary effects on galactic spectra can be observed, this will be difficult to check. Second, there may be serious troubles with stellar evolution theory (we clearly do not understand the Sun) but empirical checks with galactic clusters and the old disk (B. M. Tinsley and J. E. Gunn, unpublished) make large errors here a bit unlikely. The constraints we adopted on the age of galaxies, however, are critically dependent on the synthetic colours and could possibly be in error. Since our conclusions about the Hubble constant are affected strongly by this constraint, they should be regarded with due caution.

Dynamical evolution. It is possible that the central galaxies in clusters could undergo accretion of other cluster members through dynamical friction effects on a time scale which is sufficiently short to affect significantly the evolution of the total light. Preliminary estimates indicate that the effect is probably not important, but there are a great many uncertainties, including the question of how much of the cluster mass resides in the galaxies themselves, and whether clusters typically have a cold central subsystem from which accretion could occur rather efficiently. It seems futile to try to estimate the effect more accurately until at least these questions are answered.

Density limits. The constraint on Ω^* is weakened somewhat by the possible existence of a non-zero Λ , since the local super-cluster flow uniformity is no longer an effective argument, but the other arguments seem still quite persuasive. It is of course not clear what the status of the primaeval deuterium production constraint is, nor will it be until one can reasonably rule out subsequent production.

It is clear that if H_0 is small, the age constraint on galaxies is correct, and q_0 is large and negative, then deuterium probably cannot be primordial, Ω cannot be small, and one cannot escape the conclusion that Λ is non-zero and positive. The incorrect piece(s) of the puzzle are well hidden, and the situation is, as is usual in observational cosmology, that one awaits new and better data.

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Note added in proof: More detailed calculations by J. P. Ostriker and S. D. Tremaine (*Astrophys. Lett.*, in the press) and S. D. M. White (unpublished) show that the effect mentioned in the paragraph "Dynamical evolution" may be more important.

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Identification of two copies of the gene for the elongation factor EF-Tu in *E. Coli*

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Two copies of the structural gene for the elongation factor EF-Tu have been identified in Escherichia coli: one near rif and the other near str. The latter seems to belong to a single transcriptional unit together with the genes for ribosomal protein S7, S12 (str) and the elongation factor EF-G (fus).

PROTEIN chain elongation factors, EF-G, EF-Tu and EF-Ts, have been characterised as components required for protein synthesis in *Escherichia coli* (for reviews, see refs 1-3). In addition to their role in protein synthesis, both EF-Tu and EF-Ts are known to be components of Q β -replicase⁴. Furthermore, EF-Tu has been suggested to function as a positive regulatory factor for the transcription of rRNA genes^{5,6}. Since these protein chain elongation factors, especially EF-G and EF-Tu, function in close association with the ribosome, one could reasonably expect that their amounts inside a cell would be regulated coordinately with the amount of ribosomes. Such coordinated regulation has been observed for EF-G and EF-Ts (refs 7 and 8). No study has, however, been reported on the regulation of EF-Tu (see ref. 8).

To study the regulation of synthesis of the elongation factors, information on the organisation of the genes for these factors is essential. The gene for EF-G has been identified and mapped^{9,10} at 64 min near the *str* locus that codes for ribosomal protein (r-protein) S12, but no information was available concerning the genes for EF-Tu and EF-Ts. In this paper, we report the identification of the gene for EF-Tu. It was found that there are at least two copies of the gene for EF-Tu on the haploid chromosome of *E. coli*; one is located close to the *str* gene at 64 min and the other is at 79 min close to the *rif* gene which codes for the β -subunit of RNA polymerase¹¹. In addition, the former gene seems to belong to a single transcriptional unit together with the genes for EF-G (*fus*), r-protein S12 (*str*), and S7.

λ *fus2* and λ *rif*^{d18} carry EF-Tu genes

Several λ transducing phages carrying many r-protein genes from the *str-spc* region of the *E. coli* chromosome at 64 min have been isolated previously¹¹. One of them, λ *fus2*, carries about 30 r-protein genes, and in addition, the *fus* gene which codes for EF-G. In addition to the *str-spc* region, several 50S r-protein genes are also located near *rif* at 79 min. It has been found^{12,40} that λ *rif*^{d18}, originally isolated by Kirschbaum and Konrad¹³, carries several 50S r-protein genes in addition to the genes for RNA polymerase subunits β and β' (ref. 14). Because of the close relationship between elongation factors and ribosomes, we have investigated the possibility that genes for EF-Tu and EF-Ts might be located close to the r-protein genes carried by λ *fus2* (or λ *rif*^{d18}) as is the case with the *fus* gene.

First, ultraviolet-irradiated *E. coli* cells were infected with purified transducing phages, and proteins synthesised after phage infection were labelled with ³⁵S-methionine and analysed

(see ref. 11, and the legend to Fig. 1). Ultraviolet irradiation damages bacterial DNA and decreases the synthesis of RNA and protein to a negligible level without serious damage to the RNA and protein synthesis machinery. Thus, large stimulation in the synthesis of any protein observed after introduction of intact transducing phage DNA is a strong indication of the presence of a structural gene for that protein on the transducing phage DNA. A λ lysogen was used to prevent the expression of λ genes on the infecting transducing phages. The proteins synthesised after infection with the transducing phages were analysed by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis¹⁵ followed by autoradiography (Fig. 1).

It can be seen that λ *fus2* stimulated the synthesis of several proteins. Comparison to the reference proteins indicated the presence of a radioactive protein band corresponding to the EF-G band as expected. The presence of EF-G in this band was confirmed by tryptic peptide analysis (data not shown). In addition, we found radioactive protein bands with the mobilities of the reference EF-Tu (see below) and the reference RNA polymerase subunit α (S.R.J., R.R.B., and M.N., unpublished). In contrast to λ *fus2*, neither λ *spc2* nor λ *spc1* stimulated the synthesis of the proteins corresponding to EF-G and EF-Tu. λ *spc2* and λ *spc1* are other transducing phages carrying r-protein genes including *spc*, but not *fus* (see ref. 11 and Fig. 5). To our surprise, however, we found that λ *rif*^{d18} also stimulated the synthesis of a radioactive protein which migrated like EF-Tu on the SDS-gel system used.

The radioactive proteins with the mobility of EF-Tu were further characterised. These proteins were synthesised after λ *fus2* or λ *rif*^{d18} infection in the presence of ³⁵S-methionine. We call them λ *fus2*-EF-Tu and λ *rif*^{d18}-EF-Tu, respectively. The following observations were made. (1) As described above, both λ *fus2*-EF-Tu and λ *rif*^{d18}-EF-Tu showed the same mobility in the SDS-polyacrylamide gel electrophoresis as the reference EF-Tu (see Fig. 1b). (2) Both proteins also showed the same mobility in another polyacrylamide gel electrophoresis system (pH 8.7, in 8 M urea) as the reference EF-Tu (data not shown). (3) Both λ *fus2*-EF-Tu and λ *rif*^{d18}-EF-Tu labelled with ³⁵S-methionine were purified by SDS-polyacrylamide gel electrophoresis and then digested with trypsin. The resultant methionine-containing peptides were compared with those obtained from a reference EF-Tu purified from cells labelled with ³⁵S-methionine. Radioactive methionine containing tryptic peptides were analysed by paper electrophoresis at pH 3.6. As Fig. 2 shows, both radioactive proteins, λ *fus2*-EF-Tu and λ *rif*^{d18}-EF-Tu, in fact showed a tryptic peptide pattern identical to that of reference EF-Tu. (4) Both radioactive proteins reacted with a specific antiserum against purified EF-Tu, but not with an antiserum against EF-G (data not shown). From these results, we conclude that both λ *fus2* and λ *rif*^{d18} stimulated the synthesis of EF-Tu in the ultraviolet-irradiated *E. coli* cells.

We have also demonstrated that the DNAs from λ *fus2* and λ *rif*^{d18} are both active in the synthesis of EF-Tu in an *in vitro* DNA-dependent protein synthesising system described previously¹⁶. As described in Fig. 3, radioactive proteins synthesised

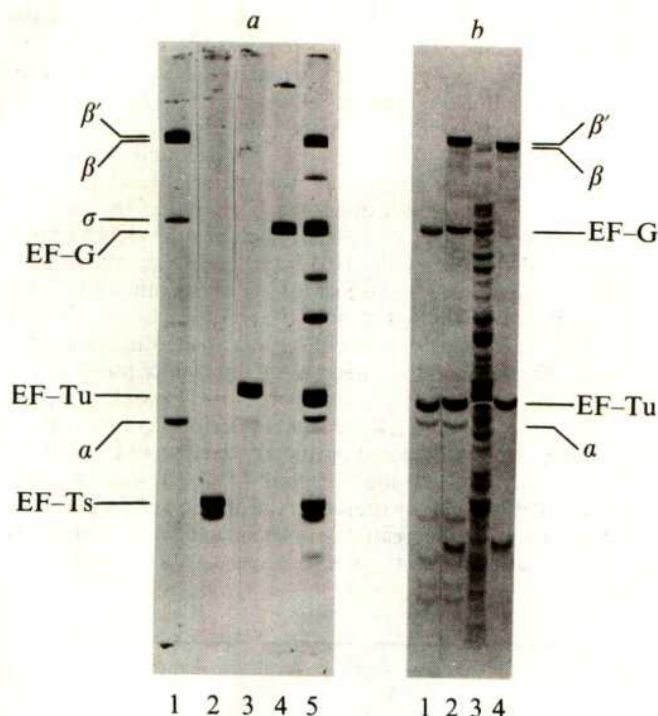


Fig. 1 Electrophoresis of proteins synthesised in ultraviolet-irradiated bacteria after infection with transducing phage. *a*, Reference proteins were electrophoresed on 8.75% polyacrylamide disc gels containing 0.1% sodium dodecyl sulphate (SDS) as described previously¹⁵. The samples were: (1) RNA polymerase; (2) EF-Ts; (3) EF-Tu; (4) EF-G; (5) a mixture of RNA polymerase, EF-Ts, EF-Tu, EF-G and several molecular weight markers. Gels were stained with Coomassie brilliant blue. The bands are identified on the left. *b*, Infection of ultraviolet-irradiated *E. coli* cells with transducing phages was performed essentially as described previously¹¹. The host was a λ lysogen of *E. coli* K12 strain 159 (ref. 32). The culture medium was a synthetic minimal medium³³ containing maltose (0.4%) and B1 (1 μ g ml⁻¹). The multiplicity of infection was approximately 10. For each sample, 5 μ Ci of ³⁵S-methionine (323 Ci mmol⁻¹) was added to a 2.5 ml culture 30 min after infection. After a 10 min labelling period and a 1-min chase with non-radioactive methionine (1 mg ml⁻¹), the culture was diluted with 5 ml of ice-cold minimal medium without maltose. The cells were pelleted, washed once with 0.05 M Tris-HCl (pH 7.9), and stored at -20 °C. Cell pellets were resuspended in 250 μ l SDS-gel sample buffer [3% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 0.063 M Tris-HCl pH 6.8], heated for 2 min at 90 °C, and 25 μ l electrophoresed in parallel with the reference proteins shown in (*a*). The stained and destained gels were sliced longitudinally, dried down under vacuum on to a piece of filter paper, and subjected to autoradiography using Kodak No-Screen X-ray film. The autoradiogram shown was exposed for 4 d. The samples were ultraviolet-irradiated cells infected with: (1) λ fus2; (2) λ rif^d18. Gel 2 contained a 1:1 mixture of the samples applied to gels 1 and 4. The sample for gel 3 was uninfected non-irradiated cells labelled with ³⁵S-methionine. The ultraviolet-irradiated cells infected with λ c1857S7 did not show any radioactive band (compare sample 1 in Fig. 4).

in vitro using λ fus2 DNA and λ rif^d18 DNA as template were first analysed by SDS-polyacrylamide gel electrophoresis. The radioactive proteins with the mobility of EF-Tu were detected in both cases. These proteins were eluted from the gels and examined for their reaction to specific antisera against EF-Tu, EF-G and r-protein L1, respectively. As shown in Fig. 3, radioactive proteins synthesised with λ fus2 DNA and λ rif^d18 DNA as template both reacted with the anti-EF-Tu serum but not with the other two antisera used. These experiments demonstrate that a protein very similar or identical to the reference EF-Tu was synthesised using either λ fus2 DNA or λ rif^d18 DNA as template. From all these experimental results, we conclude that both λ fus2 and λ rif^d18 carry a structural gene for EF-Tu.

In preliminary experiments, we have attempted to find a gene for EF-Ts on either the λ fus2 or λ rif^d18 chromosome, but with negative results.

Transcriptional unit containing *str*, *fus* and an EF-Tu gene

We have isolated several mutants of λ fus3 that have a decreased expression of the EF-Tu gene in ultraviolet-irradiated cells. λ fus3 is a derivative of λ fus2 which carries a *str*⁺ allele instead of the *str*⁻ allele present on λ fus2. We started from a lysogen (NO1380) carrying λ fus3 and with chromosomal markers *recA*⁻, *trkA*⁻, *spc*⁺, *str*⁺ and *fus*⁺. Since the *spc*⁺, *str*⁺ and *fus*⁺ alleles carried by λ fus3 are dominant over the corresponding resistant alleles on the chromosome, the phenotype of the lysogen is sensitive to all three antibiotics, that is, streptomycin, spectinomycin and fusidic acid (Str-S, Spc-S and Fus-S). We selected spontaneous Str-R mutants on media with a low potassium concentration and containing Str. By using media with a low potassium concentration, we avoided selecting mutants that had simply lost the transducing phage since the *trkA*⁺ gene on λ fus3 is required for growth on low potassium media¹⁷. Any mutation which inactivates the expression of the

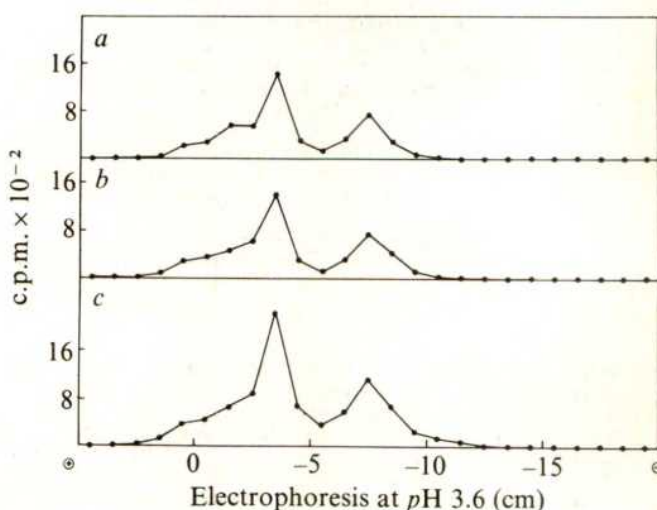


Fig. 2 Electrophoresis of ³⁵S-methionine tryptic peptides of EF-Tu. The three samples containing ³⁵S-methionine labelled EF-Tu described below were electrophoresed with 1 μ g pure EF-Tu as described in Fig. 1. The stained band corresponding to EF-Tu sized protein was cut out from each gel, washed in water, crushed, equilibrated with 0.1 M NH₄HCO₃, and incubated for 24 h at 37 °C with 100 μ g ml⁻¹ TPCK treated trypsin. Solutions were separated and the gel particles were again treated with trypsin. The solutions from the two trypsin treatments were pooled. The peptides eluted into the solution were recovered by lyophilisation and electrophoresed on Whatman 3 MM, 25 V cm⁻¹, for 2.5 h with H₂O, glacial acetic acid, pyridine (90:10:1) at pH 3.6. The origin is at 0. Strips (1-cm) were counted by liquid scintillation counting. The samples were: *a*, The EF-Tu sized protein synthesised in ultraviolet-irradiated cells after infection with λ rif^d18; *b*, the EF-Tu sized protein synthesised in ultraviolet-irradiated cells after infection with λ fus2; *c*, reference EF-Tu. The source of the reference EF-Tu was as follows. First, a partially purified EF-T preparation was made from uninfected non-irradiated *E. coli* cells [strain 159(λ)] grown in the presence of ³⁵S-methionine. The purification was done according to the previously described procedures^{34,35} as modified by W. A. Strycharz (personal communication). The cells were broken with alumina and a S100 supernatant was treated with protamine sulphate and fractionated with ammonium sulphate and isopropanol³⁴. The protein fraction containing EF-T was applied to a DEAE-Sephadex column³⁵. The column was first washed with 0.15 M KCl; EF-T was then eluted with 0.30 M KCl. The partially purified EF-T preparation thus obtained was subjected to electrophoresis on an SDS-polyacrylamide gel. The electrophoresis separated EF-Tu from Ts and all other contaminating proteins in the EF-T preparation. Pure EF-Tu prepared in this way was used as the reference EF-Tu.

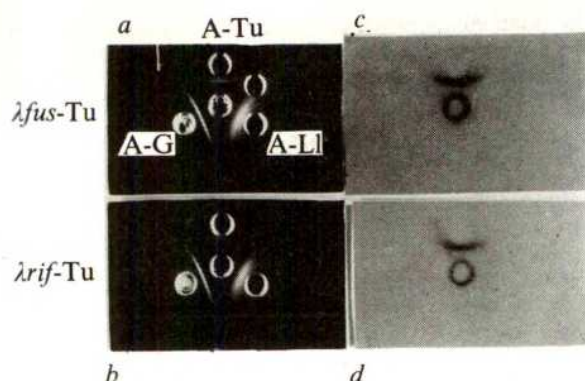


Fig. 3 Identification by radioimmunodiffusion of EF-Tu synthesised *in vitro*. ^{35}S -methionine-labelled protein was synthesised in a DNA-dependent cell-free system, using DNA from λfus2 ($35 \mu\text{g ml}^{-1}$) and that from λrif^{18} ($40 \mu\text{g ml}^{-1}$) as template. The method was similar to those described previously^{11,16}. After incubation at 37°C for 90 min, samples were treated with RNase ($10 \mu\text{g ml}^{-1}$) and DNase ($10 \mu\text{g ml}^{-1}$) at 37°C for 10 min. The resultant samples ($60 \mu\text{l}$ each) were mixed with $200 \mu\text{l}$ of the SDS-gel sample buffer (see Fig. 1), heated at 95°C for 5 min, cooled and dialysed against 4 changes of 50 ml of the SDS-gel sample buffer¹⁸. The samples were then subjected to the SDS-polyacrylamide gel electrophoresis as described in Fig. 1 and the radioactive products were detected by autoradiography. The radioactive proteins which had mobilities identical to reference EF-Tu were eluted from the sliced gels. This was done by crushing the gels and incubating them with 1.5 ml of $0.1 \text{ M NH}_4\text{HCO}_3$ ($\text{pH} \sim 8.0$) for 20 h at 37°C . Bovine serum albumin ($50 \mu\text{g}$) was added to the eluates and the mixtures were lyophilised. Lyophilised proteins were then dissolved in $50 \mu\text{l}$ of Tris-HCl (0.03 M , $\text{pH} 7.4$)-MgCl₂ (0.02 M)-KCl (1 M)-2-mercaptoethanol (0.006 M) buffer containing the following carrier proteins: purified T (EF-Tu and EF-Ts), $3 \mu\text{g}$; purified EF-G, $2 \mu\text{g}$; unfractionated 50S ribosomal proteins, $30 \mu\text{g}$. These protein mixtures containing radioactive proteins were analysed by immunodiffusion as described before^{16,36}. The samples were placed in the centre wells and antisera against EF-Tu ("A-Tu"), EF-G ("A-G") (both gifts from H. Weissbach) and 50S protein L1 ("A-LI")¹² were placed as indicated in the figure (both in a and b). Each set of pictures shows a gel (a and b) and an autoradiogram of the dried gel (c and d). The results obtained with the sample containing the *in vitro* product using λfus2 DNA as template are shown in a and c and those obtained using λrif^{18} DNA are shown in b and d. It can be seen in both cases that only the Tu-anti-Tu precipitin bands contained radioactive proteins and the other precipitin bands did not.

str^s gene on λfus3 or changes it to a *str^r* allele will lead to the Str-R phenotype. Among 25 independent Str-R mutant lysogens isolated, 15 were Fus-R (but not Spc-R). Transducing phages were isolated and characterised. Phages that seemed to have a mutation inactivating the *str^s* and *fus^s* genes were further analysed for their ability to stimulate the synthesis of EF-G, EF-Tu and r-proteins. Table 1 and Fig. 4 give the results obtained with two mutant transducing phages, $\lambda\text{fus3-}\Delta 101$ and $\lambda\text{fus3-I103}$. As can be seen, these two mutations substantially reduce the expression of the genes for EF-G, EF-Tu, S12 (*str*), and S7. It should be noted that the expression of these genes does not seem to be completely eliminated. For example, a small amount (about 3% of the parent) of EF-G seems to be made from $\lambda\text{fus3-}\Delta 101$ (see Fig. 4). This suggests that the inactivation of some or all of these genes is not a direct inactivation such as that caused by a deletion.

The mutations carried by these two transducing phages, $\Delta 101$ and I103, were characterised by the density of the mutant phages and by electron microscopic analysis of heteroduplexes of the mutant phage DNAs and several other phage DNAs including the λfus2 DNA. We have found that mutation I103 is an insertion of a small DNA with the size of IS2 (refs 18–20) and is located close to the right end of the bacterial genome carried by λfus3 (see Fig. 5). The distance between the site of the insertion and the right *coli-λ* junction was found to be 0.60 ± 0.10 kilobase (kb) in length. Mutation $\Delta 101$ was found to be a

small deletion which is also located near the right end of the λfus3 chromosome. This deletion removes the *coli-λ* junction; it deletes about 0.3 kb of λ DNA together with about 1.3 kb of the right end of the bacterial DNA (Fig. 5). The sum of the minimum probable sizes of each of the inactivated genes, that is, the structural genes for EF-G, EF-Tu, S12 and S7, can be calculated from the molecular weights of these proteins. The sum is 4.6 kb in length, and is much larger than the bacterial DNA removed by $\Delta 101$ or the space between the right "*coli-λ*" junction and the site of the I103 insertion. Thus, most of the inactivated genes seem to be located left of the mutations $\Delta 101$ or I103. We conclude that most of the genes for EF-G, EF-Tu, S12 (*str*) and S7 are not deleted, but inactivated by some kind of polar effect. Since the direction of the transcription in this region is from right to left²¹, and there is no known λ promoter on the λ DNA present on λfus3 , the transcription of these genes seems to be from a bacterial promoter which would be located near the right end of the bacterial DNA. Thus, the polar effect of $\Delta 101$ is probably caused by the deletion of the promoter or some other essential regulatory elements for these inactivated genes. Similarly, inactivation of these genes by I103 is probably

Table 1 Relative synthesis of protein in ultraviolet-irradiated bacteria after infection with λfus3 , $\lambda\text{fus3-}\Delta 101$, $\lambda\text{fus3-I103}$

Protein	Transducing phage		
	λfus3	$\lambda\text{fus3-}\Delta 101$	$\lambda\text{fus3-I103}$
EF-G	1.0	0.03	0.19
EF-Tu	1.0	0.11	0.23
S7	1.0	0.31	0.34
S12	1.0	0.03	0.13
Others	1.0	1.02	0.99

Ultraviolet-irradiated bacteria were infected with $\lambda\text{cI857S7}$, λfus3 , $\lambda\text{fus3-}\Delta 101$ and $\lambda\text{fus3-I103}$ as described in Fig. 1. The relative amounts of EF-G and EF-Tu synthesised after phage infection were determined from the autoradiogram shown in Fig. 4. The autoradiogram was scanned with a Joyce-Loebl microdensitometer and the amounts of EF-G and EF-Tu synthesised relative to the amount of RNA polymerase subunit α synthesised were calculated from the areas of the peaks. The synthesis of α does not seem to be affected by the $\Delta 101$ and I103 mutations. Its structural gene is between *trkA* and *spc* (S.R.J., R.R.B. and M.N., unpublished). The amount of each protein synthesised was then normalised to the amount synthesised after infection with the parent phage λfus3 . The relative synthesis of S7 and S12 was determined from separate experiments in which the labelling and sample preparation were done as described previously²¹. The bacterial host for the experiment was prelabelled with ^{14}C -leucine, irradiated, and infected with transducing phages. Proteins synthesised after infection were labelled with ^3H -leucine and electrophoresed on a two-dimensional polyacrylamide gel that separates r-protein³⁰. The $^3\text{H}/^{14}\text{C}$ ratio for all the r-proteins except S1, L31 and L34 was determined. The stimulation of each r-protein *i* was calculated, $S_{rpi} = (^3\text{H}/^{14}\text{C})_{\text{transducing phage infected}} / (^3\text{H}/^{14}\text{C})_{\lambda\text{cI857S7 infected}}$. The stimulation of the synthesis of each r-protein relative to the synthesis of S4 was then determined by dividing S_{rpi} by the stimulation of the synthesis of S4, S_{rpi}/S_{rps4} . The structural gene for S4 is between *trkA* and *spc* (refs 21 and 31) and its synthesis does not seem to be affected by the $\Delta 101$ and I103 mutations. The relative stimulation of the synthesis of each protein by $\lambda\text{fus3-}\Delta 101$ and $\lambda\text{fus3-I103}$ was then normalised to the relative stimulation by λfus3 : $(S_{rpi}/S_{rps4})_{\text{transducing phage infected}} / (S_{rpi}/S_{rps4})_{\lambda\text{fus3 infected}}$. Data for S7 and S12 are presented here. The stimulation of the synthesis of some protein that comigrates with L20 is also reduced to less than 10% by $\Delta 101$ and I103. Since some S12 comigrates with L20 (ref. 30 and M.N., unpublished), it seems likely that the apparent stimulation of the synthesis of L20 by λfus3 and its reduction by $\Delta 101$ and I103 is due to the presence of S12 in this spot. The stimulation of the synthesis of each of the other r-proteins whose genes appear to be on λfus3 (27 proteins) was not significantly affected. The average of the normalised relative stimulation for these proteins is given in the bottom line. We previously observed an apparent weak stimulation of the synthesis of S7 in ultraviolet-irradiated cells after infection with λspc1 and λspc2 (ref. 11). The stimulation of the synthesis of S7 is, however, much greater with λfus3 or λfus2 (S.R.J. and M.N., unpublished). Also, we have only been able to synthesise S7 *in vitro* using λfus2 DNA as template and not with λspc1 or λspc2 DNA (L.L. and M.N., unpublished). Thus it is likely that the structural gene for S7 is present on λfus2 and λfus3 , but not on λspc1 or λspc2 .

due to a strong polar effect affecting distal genes that is analogous to the polar effect of IS2 studied in other systems¹⁸⁻²⁰. From these results, we conclude that the genes for EF-G (*fus*), EF-Tu, S12 (*str*) and S7 probably belong to a single transcriptional unit. The promoter for this transcriptional unit is presumably located in the region between the I103 insertion and the *coli*- λ junction, the region that is also covered by the Δ 101 deletion. More complicated models, however, such as the expression of one transcriptional unit being required for the expression of another unit, would also be consistent with present observations.

Because the mutations Δ 101 and I103 affect the four genes described above, but do not affect the remaining r-protein genes, including *spc* or the gene for the RNA polymerase subunit α , these latter genes presumably belong to another one or more transcriptional units. This is consistent with the previous conclusion that there is more than one r-protein gene transcriptional unit in the *str*-*spc* region of the *E. coli* chromosome²¹. As mentioned above, EF-Tu is synthesised in ultraviolet-irradiated cells after λ *fus*2, but not after λ *spc*1 or λ *spc*2, infection. These results suggest that the gene for EF-Tu is not on the λ *spc*2 genome, placing the gene location as shown in Fig. 5. This mapping position is consistent with the conclusion described above that the gene for EF-Tu is in the same transcriptional unit as *str* and *fus*.

EF-Tu gene in *rif* region

The EF-Tu gene has been mapped on the λ *rif*^d18 chromosome by making DNA fragments with various DNA restriction endonucleases and testing the ability of each fragment to synthesise EF-Tu in a DNA-dependent protein synthesising system (L.L., M. Yamamoto and M.N., unpublished). The order of the genes is shown in Fig. 5. We do not have any experimental information on the question of whether the EF-Tu gene is cotranscribed together with any of the neighbouring genes, the rRNA gene set¹², 50S r-protein genes^{12,40} or the genes for the RNA polymerase subunits β and β' (refs 14, 22 and 23).

Two genes for EF-Tu

We believe that the presence of the genes for EF-Tu on both λ *rif*^d18 and λ *fus*2 reflects the presence of at least two genes for EF-Tu on the normal *E. coli* chromosome. Since the gene for EF-Tu was not used as a selection marker to isolate the λ *rif*^d18 or λ *fus*2 transducing phages, it is difficult to imagine that the EF-Tu gene was accidentally incorporated into these transducing phages during construction of the phage. In addition, we have observed that another transducing phage, ϕ 80*rif*^r, isolated by Konrad, Austin and Kirschbaum²⁴, also stimulates synthesis of EF-Tu, in addition to RNA polymerase subunits β and β' , and several 50S r-proteins, in ultraviolet-irradiated cells (our unpublished experiments). It was also shown that the DNA of ϕ 80*rif*^r has a near complete homology to λ *rif*^d18 phage DNA in the substituted bacterial DNA region containing the 50S r-protein genes, the EF-Tu gene and the rRNA gene set, as judged by electron microscopic analysis of the heteroduplex formed from both the phage DNAs (G.D. Strycharz, and M.N., unpublished.) Since ϕ 80*rif*^r was originally isolated in a completely different way from that used for the λ *rif*^d18 isolation, this observation also supports the conclusion that the organisation of the genes found on the λ *rif*^d18 phage chromosome applies to the normal *E. coli* chromosome. We conclude that there are at least two genes for EF-Tu. We call the one near *str* and *fus* "tufA" and another near *rif* "tufB".

There are some reports on the isolation of mutants which have alterations in EF-Tu (refs 25 and 37). The genes responsible for the alterations have not, however, been mapped.

The significance of the presence of two genes for EF-Tu, one at the *str*-*spc* region and the other at the *rif* region, is a matter of speculation at the moment. The two chromosomal regions

where the *tuf* genes are located contain many of the genes for ribosome and RNA polymerase components. One could speculate that these two regions are physically proximate within *E. coli* cells and that the transcription and translation of these genes as well as the assembly of both RNA polymerase and ribosomes take place all in one place. It is possible that a physical interaction (by DNA sequence homology) between the two *tuf* genes is involved in maintaining a chromosome structure with the two regions in contact. A condensed *E. coli* chromosome structure with several "contact regions" has been proposed recently²⁶.

Alternatively, EF-Tu might function as a regulatory protein for the expression of its own operon. If such regulation takes place using a "nascent form" of EF-Tu and it acts only *cis* (that is, only on a regulatory element of its own operon), the presence of one EF-Tu gene for each important gene cluster, one at the *rif* region and the other at the *str*-*spc* region, would be required for the proper regulation.

It is also possible that the two observed *tuf* genes may be slightly different and may code for two kinds of EF-Tu that have slightly different primary structures and different cellular functions. It is equally conceivable that the presence of the two *tuf* gene copies may simply reflect a need for a greater amount of EF-Tu for cellular functions. In this connection, we note that although the stoichiometric relationship of both EF-G and EF-Ts to ribosomes has been analysed^{7,8,27}, no such work

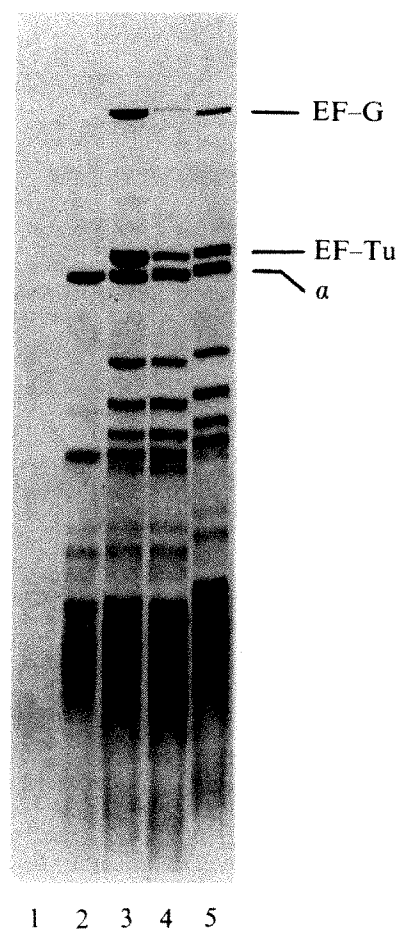
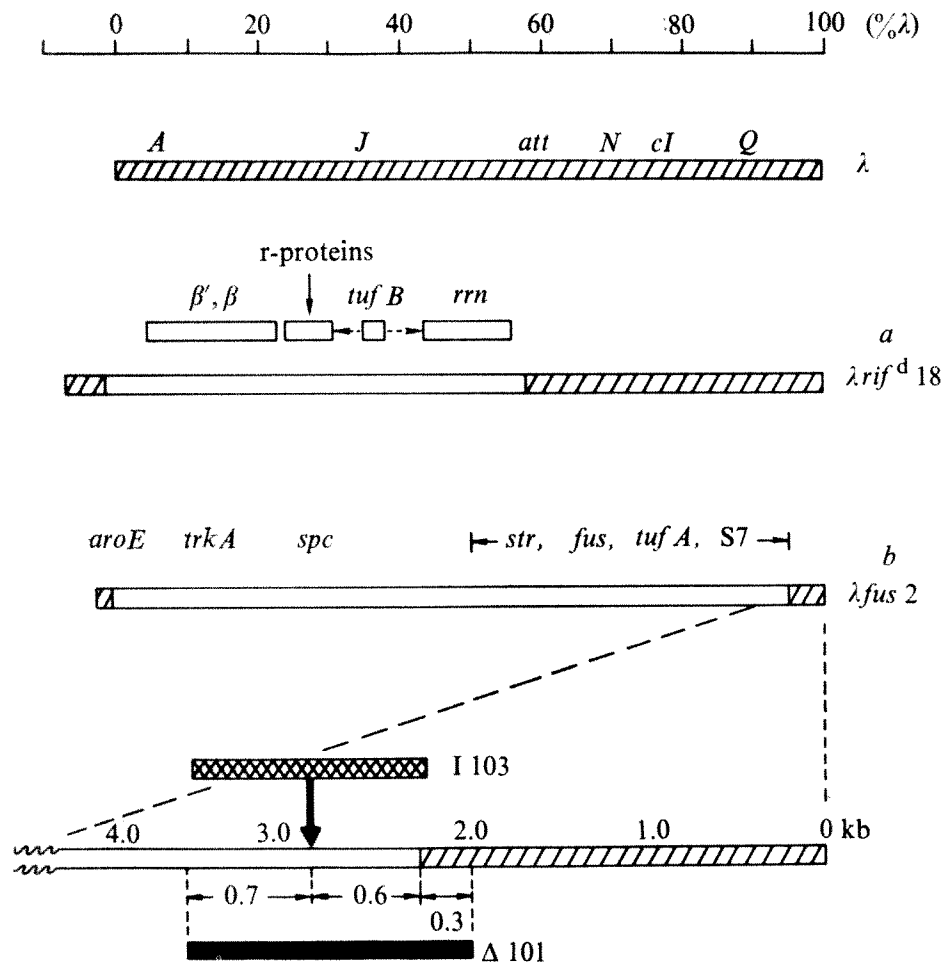


Fig. 4 Electrophoresis of proteins synthesised in ultraviolet-irradiated bacteria after infection with mutant transducing phages. Ultraviolet-irradiated cells were infected with (1) λ c1857S7, (2) λ *spc*2, (3) λ *fus*3, (4) λ *fus*3- Δ 101, and (5) λ *fus*3-I103 as described for Fig. 1. Samples were prepared and analysed by SDS-polyacrylamide gel electrophoresis as described in Fig. 1 except that the gels contained 12.5% acrylamide instead of 8.75%. Autoradiograms of dried gels are shown. Some of the bands are identified on the right.



to 48.5% λ measured from the left end of λ genome, and inserts a 50.7% λ units length fragment of bacterial DNA; λ $spc1$ deletes λ DNA from 1.9% λ to 56.8% λ and inserts 38.4% λ units length fragment of bacterial DNA; λ $fus2$ deletes λ DNA from 1.9% λ to 94.9% λ and inserts 96.5% λ units length fragment of bacterial DNA. Thus, λ $fus2$ has a homology to the DNA segment from the left end of λ $spc2$ (total about 53% λ units) and a homology to the λ DNA at the end of the molecule with the length of 5.1% λ units. The structure of these transducing phages has been determined previously (M. Fiant, W. Szybalski, F. Blattner, L.L., S. R. J. and M.N., unpublished).

has been reported with respect to EF-Tu (see ref. 8).

Finally, we have shown that the genes for EF-G (fus) and that for EF-Tu ($tufA$) probably belong to a transcriptional unit together with other r-protein genes including str . Thus we expect that both the fus gene and the $tufA$ gene are subject to the same control system as those governing r-protein genes, such as the stringent-relaxed control system^{28,29}. We do not, however, know whether the $tufB$ gene is also controlled by the same system. Thus more experiments are required to clarify the problems related to the regulation of the tuf gene expression.

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letters to nature

New radio map of Cassiopeia A at 5 GHz

THE radio source Cassiopeia A (Cas A) is believed to be the remnant of a type II supernova that occurred in our galaxy about 280 yr ago (refs 1 and 2). The radio remnant forms an irregular shell of radius $\sim 2'$, corresponding to ~ 1.6 pc at its distance of 2.8 kpc (ref. 2), which is broadly coincident with an incomplete shell of fast-moving optical knots¹. We present here a new radio map of the source with a resolution three times greater than has previously been achieved³.

During November 1974, Cas A was observed with the Cambridge 5-km telescope⁴ at a frequency of 5 GHz, giving half-power beamwidths of $2''$ in right ascension and $2.3''$ in

declination. A total of 128 interferometer spacings were used, so that the synthesised beamshape has its first grating response at $5'$ in right ascension and does not confuse the source. The effective sensitivity is determined by the general level of the side-lobes, which, from regions of the map outside the source, is estimated to have a brightness temperature of 50 K, about 1% of the peak brightness of the source. The sensitivity from thermal noise alone would be 15 K.

Figure 1 shows the map, with contours of the Stokes' parameters I - Q . Figure 2 is a radio photograph showing the finer details not visible at the contour interval (480 K) of Fig. 1. The areas of lower surface brightness are shown very clearly in this negative print, which has 128 separate grey levels.

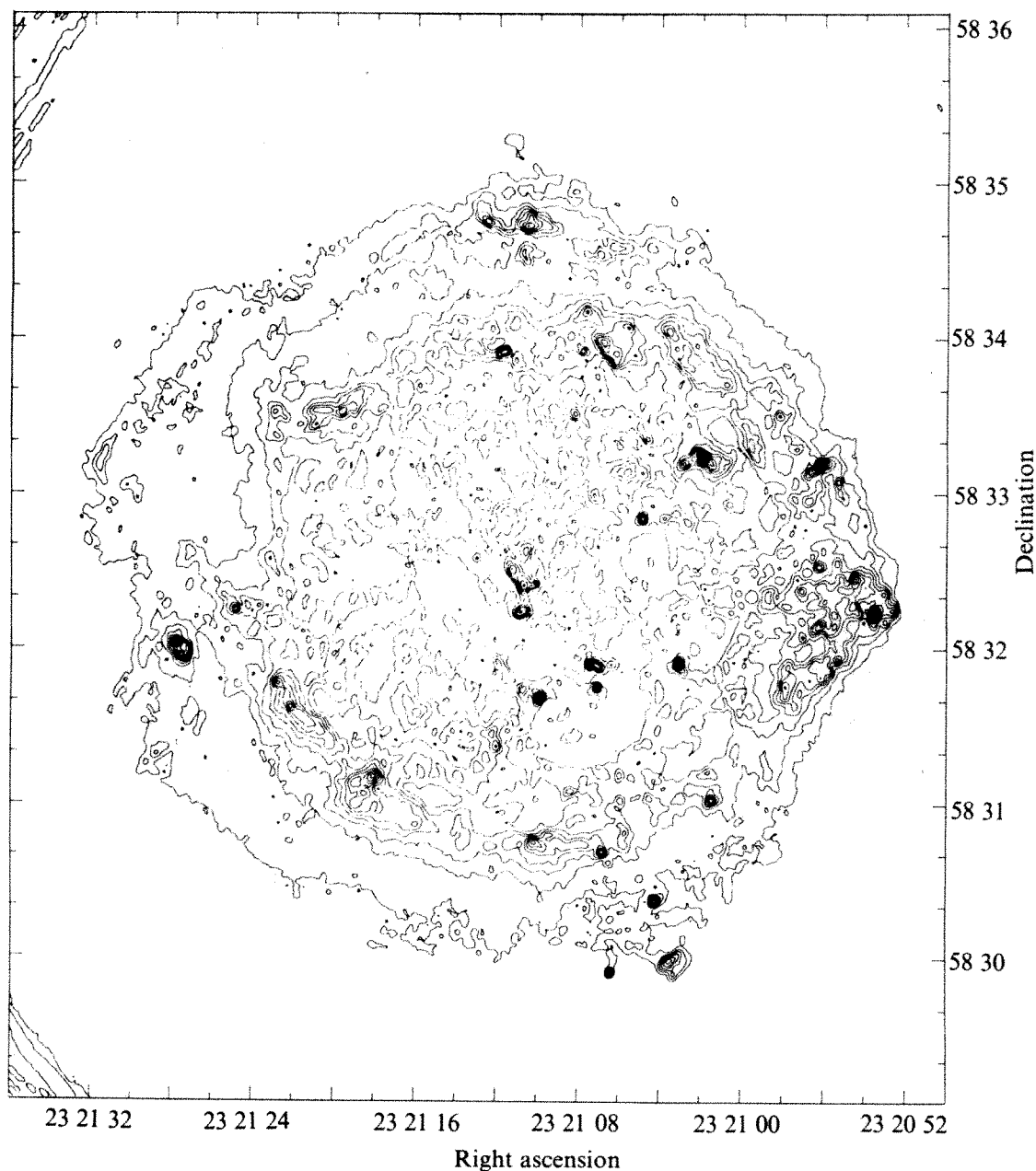


Fig. 1 Map of Cas A at 5 GHz. The contour interval is 480 K and the coordinates refer to epoch 1950.0

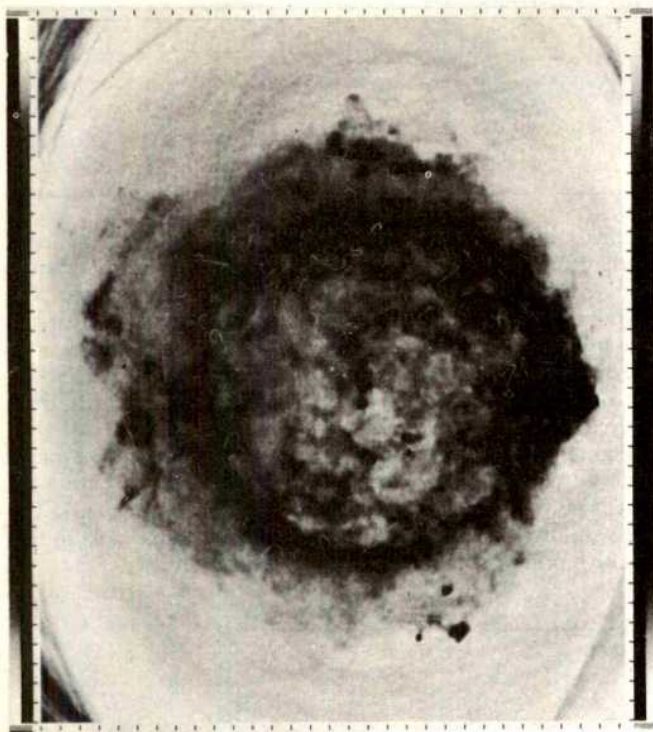


Fig. 2 Radio photograph of Cas A. The grey scales on either side are linear in radio intensity. The features visible at the corners of the map represent the first grating response.

The overall structure of the source is that of a ring, presumably the two-dimensional projection of a spherical shell. The observations show clearly that the brightest part of the ring of emission lies well inside the edge of the source, with a plateau of lower surface brightness outside, as has been noted previously³. This plateau itself shows detailed structure and in many areas seems to provide a well defined outer boundary to the remnant. The most plausible interpretation of these rather sharp boundaries is that they represent the position of a contorted, outer shock front and that relativistic electrons are able to diffuse as far as this shock. The extent of the plateau varies considerably over the remnant. In particular, there is no evidence of any plateau outside the strong peaks to the west of the source.

The ring of brightest emission shows considerable departures from circular symmetry. The well known gaps at position angle (p.a.) 70° and 210° (ref. 3) are less prominent at this resolution and are spanned by weak ridges of emission. There are several small, but clear-cut, gaps in the shell and one of these, at p.a. 200° ($\alpha = 23$ h 21 min 06.3 s, $\delta = 58^\circ 30'45''$), is of interest in relation to the bright knots of emission outside the main ring in this area. One of the new features revealed by the present observations is that the emission within the main ring shows a marked cellular structure defined by weak ridges on scales of $\sim 20''$; towards the edge of the source some of the cells seem flattened, suggestive of foreshortening. Such a cellular structure and the generally chaotic appearance of the remnant is in accord with models of young supernovae^{5,6}, in which the radio shells are first formed as a result of turbulence generated by the interaction between the ejected material and swept-up interstellar medium.

A notable feature of the remnant revealed by this map is the existence of many compact, strongly emitting regions on the scale of the beamwidth, particularly in the sector between p.a. 200° and 310°. Figure 3 is a more detailed contour map of part of the south-west region of the source and shows the proliferation of emission peaks in this area. Projection effects make it impossible to determine the position of most of these blobs relative to the main shell, but those outside

the shell to the south have clearly broken away from it. The radio photograph (Fig. 2) shows a V-shaped feature pointing towards these southern knots. Although this may merely be due to a chance coincidence of knots and filaments, it is reminiscent of a nozzle and we speculate that it represents the remains of that part of the shell from which the outer blobs detached, particularly as its axis coincides with the narrow gap in the bright ring of emission noted earlier. Another such nozzle can be picked out behind the strong, compact emission peaks at the extreme west of the source. Of the southern peaks, the small, slightly resolved, outermost knot at $\alpha = 23$ h 21 min 06.3 s, $\delta = 58^\circ 29'47''$ was not visible in 1969 (ref. 3) and it is intriguing that a recent plate taken by van den Bergh (personal communication) shows that an optical filament has now appeared in this position. This knot seems to have pulled out a neck of plateau in a non-radial direction and indeed all the outlying knots seem to have diffuse emission associated with them. There have also been many other significant changes both of flux and position since 1969 and a detailed study of these will be published elsewhere.

The minimum energy density in relativistic electrons and magnetic field required to account for the radio synchrotron emission from the most compact knots is $\sim 8 \times 10^{-8} \text{ J m}^{-3}$, equivalent to a magnetic field of $3 \times 10^{-7} \text{ T}$. If these knots are to be confined by the thermal pressure of hot, swept-up interstellar gas, then the product $n_H v_3^2$ must be greater than 14, where n_H is the local particle density in the interstellar medium (atom cm^{-3}) and v_3 is the shock velocity (units of $1,000 \text{ km s}^{-1}$). Typical velocities of the fast optical filaments

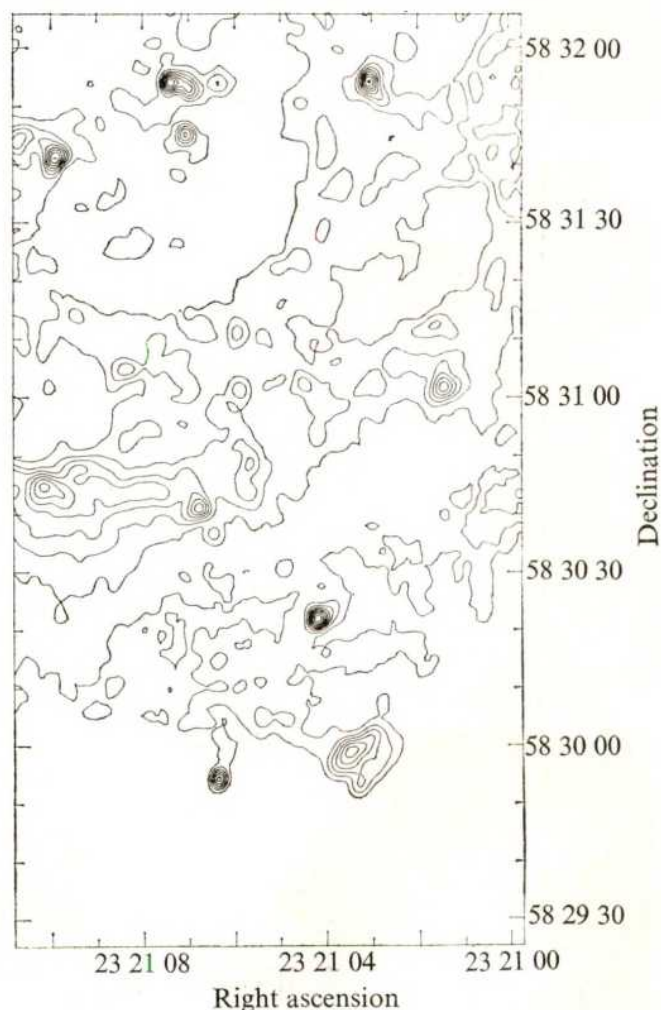


Fig. 3 Contour map of part of the south-west quadrant of Cas A. The contour level is 480 K.

are $5,000\text{--}7,000\text{ km s}^{-1}$, so containment is not difficult for reasonable values of n_H . Such high pressures, however, imply the existence of a very efficient mechanism for the production of magnetic field.

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Observations at 6 cm of the solar active region

PRECISE knowledge of the fine structure of radio sources in solar active regions, and especially of their angular size and brightness temperature is important for the gyro-resonance absorption theory of the slowly varying component. In particular, such information is important for verifying proposed models^{1–3} of resonance absorption layers above sunspots for which measurement of circular polarisation is also extremely useful. The active regions at centimetre wavelengths remain stable in intensity and structure for periods of ~ 12 h. Consequently, the existing long baseline interferometers can be used to obtain synthesised maps from observations over those periods. Our first attempt to obtain such maps was in 1973 with the National Radio Astronomy Observatory interferometer at wavelengths of 3.7 and 11.1 cm. These maps were not very clean because of the availability of only three

baselines⁴, a problem which does not arise with the Westerbork synthesis radio telescope, which has twenty baselines. Here we describe the preliminary results of synthesis of a solar active region observed on May 8, 1974 from results obtained on May 8–10, 1974 with the Westerbork synthesis radio telescope (WSRT) in Holland⁵ at a wavelength of 6 cm.

The WSRT consists of twelve 25 m paraboloids along an east–west baseline, combined to form 20 interferometers. For our observations the minimum spacing was 90 m, the maximum 1,458 m, the separation between successive baselines was 72 m and the integration time was 30 s. At the wavelength of 6 cm and for a source with $\delta = 17^\circ$ the WSRT has a synthesised beam size of $6.3''$ (E–W) \times $21.5''$ (N–S) and a primary beam with half width of $10'$. For a single 12-h period of observation the first grating ring has a radius of $3'$ (E–W) and $10'$ (N–S). In spite of the presence of several sources in the field of view, the associated grating rings were successfully removed from the maps by using conventional 'cleaning' techniques⁶. Because baselines smaller than 90 m are missing, the instrument eliminates a considerable fraction of the flux of sources with size greater than $1'$ (E–W) \times $3'$ (N–S).

There are two major problems with a sidereal instrument used for solar observations: appropriate source tracking and receiver saturation. As the WSRT tracks at sidereal rate, we had to update the pointing of the dishes every 30 min. The difference between solar and sidereal motion over this interval (30 min) is about $0.6'$, which is comparable with the pointing error of the dishes. The phase drift of the visibility function due to solar motion was corrected in the data processing stage by a standard WSRT reduction routine. The accuracy of the tracking obtained this way is better than the synthesised beam width. Because of perspective changes caused by solar rotation during the 12-h observing period, however, only the region near the centre of the field of view can be tracked accurately. To avoid receiver saturation, an extra attenuation of about 25 dB was placed in front of the correlators. This attenuation was removed during calibration. The input of each correlator was adjusted to a constant level, the same for all correlators and all observations (solar and calibrator).

The instrument was used for 12 h of solar observation and 12 h of calibration each day. The sources 3C286, 3C147 and 3C48 were used for phase calibration and 3C147 for amplitude calibration. The accuracy of the phase calibration is better

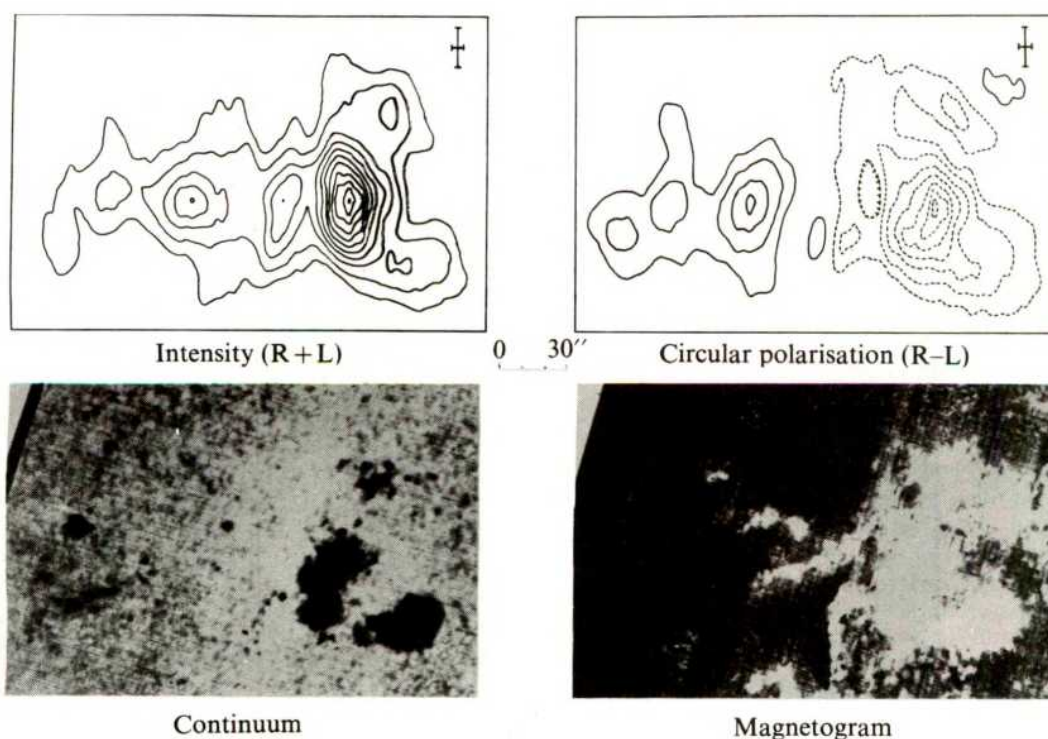


Fig. 1 Total intensity and circular polarisation maps of an active region near S12W05 on May 8, 1974. The beam size is shown in the upper right-hand corner. Contour levels are: 0.1, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50 ($\times 10^6$ K) for $R+L$; $-0.750, -0.625, -0.500, \pm 0.375, \pm 0.250, \pm 0.125, \pm 0.050$ ($\times 10^6$ K) for $R-L$. Negative contours are shown by broken contour lines. The hatched contour on the $R-L$ map shows a decrease of the absolute value of the polarisation. Continuum photograph and magnetogram courtesy Sacramento Peak Observatory, AFCRL. Positive magnetic field is white.

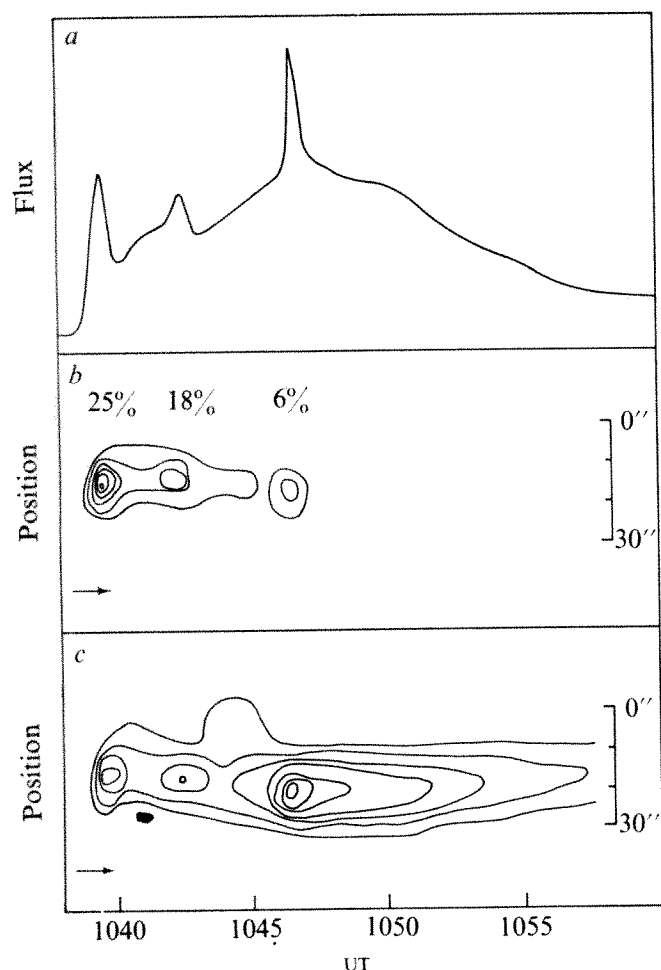


Fig. 2 A burst observed on May 9, 1974 with the WSRT: *a*, Flux (arbitrary units) as observed with a single Westerbork dish. *b*, Contours of equal circular polarisation (L-R) as a function of east-west position and time. Contour levels are: 0.29, 0.58, 0.87, 1.16, 1.45 and $1.74 (\times 10^7 \text{ K arc s})$. The peak values are 1.85×10^7 , 1.20×10^7 and $0.73 \times 10^7 \text{ K arc s}$ for the first, second and third peak respectively. The numbers indicate the percentage polarisation of the peaks. *c*, Equal intensity contours (L+R) as a function of east-west position and time. Contour levels are: 1.7, 3.4, 5.1, 6.8, 8.5, 10.2 and $11.9 (\times 10^7 \text{ K arc s})$. The peak values are 7.1×10^7 , 6.9×10^7 and $12.1 \times 10^7 \text{ K arc s}$ for the first, second and third peak respectively. The arrows in *b* and *c* show the position of the associated active region at S07W37. The response of constant sources has been subtracted from *b* and *c*.

than 1° , and the amplitude calibration is accurate to about 4%. The amplitude error is higher than the customary 1% in other WSRT observations because of the extra attenuation.

The data were calibrated and Fourier transformed by using standard WSRT reduction programs. The resulting 'dirty' maps were cleaned of sidelobes at the University of Maryland. All four Stokes parameters were observed, but the linear polarisation maps have a very noisy appearance, which puts the linear polarisation limit at about 2%. A total intensity and circular polarisation map from May 8 is shown in Fig. 1, together with a white light photograph and a magnetogram from Sacramento Peak Observatory. The coordinates of the sunspot group at 0 UT were S12W05. This group is part of McMath region 12906, which had a very extended plage.

Several individual sources show up in both total intensity and circular polarisation maps. The brightest source with a peak temperature of $2.5 \times 10^6 \text{ K}$ corresponds to one of the two large sunspots. The emission is extended northwards to the group of small sunspots, south-west towards the other large sunspot and eastward towards another group of small sunspots. The other three sources are overlying the plage with the brightest component located close to the neutral

line of the magnetic field. A low brightness halo surrounds the sources. The halo has a brightness temperature of about 10^5 K . It does not, however, extend over the entire plage region. This is probably because the interferometer spacings less than 90 m were not available for our observation.

As shown in the magnetogram, the active region has a bipolar structure with positive polarity concentrated on the main sunspots and negative polarity scattered over the plage and some minor sunspots. H α photographs show filaments along the neutral line. The circular polarisation map closely follows this configuration, with regions of positive magnetic polarity having the strongest degree of left circular polarisation. It is interesting to note that the source just to the east of the main peak lies along a neutral line and has a bipolar circular polarisation structure.

During our three days of observations several bursts were recorded. Because of the short lifetime of the bursts, it is not possible to produce a two-dimensional map with the interferometer. But, as the 20 baselines of the WSRT are located along a straight line, it is easy to combine them and produce the equivalent of a fan beam scan. This one-dimensional intensity distribution is displayed in Fig. 2 for a burst observed on May 9. We have used a contour map display, with the intensity shown as a function of position and time. For the sake of clarity we have subtracted the response of the constant sources. Our resolution is about $6''$ in the east-west direction; the integration time is 30 s. Figure 2 also gives the flux of the burst as recorded by a single Westerbork dish.

This particular event corresponds to an H α flare⁶ of importance 1B at S05W38. The flare occurred near a sunspot group shown by an arrow in Fig. 2. The burst shows three distinct maxima, the third and brightest of which coincides in time with the reported optical maximum. The east-west size of the burst during this maximum is $12''$; assuming the same north-south size we obtain a peak brightness temperature of 10^7 K . This particular peak is about 6% left circularly polarised. The other two peaks have lower brightness temperatures but are more strongly left circularly polarised; the polarisation is of the same sense as that of the associated active region. The position of the maximum emission changes with time, which cannot be due to the change of baseline because this change is very small, indicating that the position of the burst changes during its lifetime.

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Special relativity and very long baseline interferometers

WHEN baseline lengths approach the diameter of the Earth, relativistic effects become important, especially in geodetic applications. Failure to take account of these effects results in errors that are equivalent to clock errors of the order of a few parts in 10^{12} .

One method of making the correction has been to use a geocentric coordinate system for the purpose of working out the classical Doppler shift and then to multiply this by a time-dilatation term involving the difference in axial distances between the two receivers. This procedure leads to an erroneous result¹.

The correct approach involves the use of two coordinate systems. One is the source frame of reference and the other is the frame of reference of one of the receivers. To avoid repetition, a table of symbols with their definitions is given in Table 1. The symbols (U) and (O) in the right hand column denote unobservable and observable quantities, respectively. The object of the calculations presented here is to recast an equation involving unobservables into one containing only observables.

Table 1 Definition of symbols

Symbol	Definition
\mathbf{v}_1	(U)* Velocity of receiver 1 relative to source (Source frame)
\mathbf{v}_2	(U) Velocity of receiver 2 relative to source (Source frame)
\mathbf{l}	(U) Unit vector in direction of Earth as seen from source (Source frame)
\mathbf{l}_1'	(O)* Unit vector in direction of the source as seen from receiver 1 (Receiver 1 frame)
\mathbf{l}_2'	(O) Unit vector in direction of the source as seen from receiver 2 (Receiver 2 frame)
α_1	(U) Angle between \mathbf{v}_1 and \mathbf{l} (Source frame)
α_2	(U) Angle between \mathbf{v}_2 and \mathbf{l} (Source frame)
α_1'	(U) Angle between \mathbf{v}_1 and \mathbf{l}_1' (Receiver 1 frame)
\mathbf{V}	(O) Velocity of receiver 2 relative to receiver 1 (Receiver 1 frame)
ϕ_1'	(O) Angle between \mathbf{l}' and \mathbf{V} (Receiver 1 frame)
ϕ_2'	(O) Angle between \mathbf{l}_2' and \mathbf{V} (Receiver 2 frame)
ν	(U) Frequency emitted by source
ν_1'	(O) Frequency received at receiver 1
ν_2'	(O) Frequency received at receiver 2
c	Velocity of light

*U, Unobservable and O, observable quantities.

Except for \mathbf{V} , the velocity of receiver 2 relative to receiver 1, unprimed quantities are in the source frame of reference, and thus unobservable, and primed quantities are in the frame of reference of receiver 1. The receivers need not be Earth-bound, but the source and receiver frames are treated as inertial systems.

Following Pauli², the source coordinate axes are arranged so that the x axis is in the direction of \mathbf{v}_1 . The y axis is in the plane of \mathbf{v}_1 and \mathbf{l} and the z axis is perpendicular to x and y . The coordinate axes of receiver 1 are parallel to the source axes, but the two systems are in uniform motion relative to one another. The basic equations to be considered are:

$$\nu_1' = \nu \frac{1 - (v_1/c) \cos \alpha_1}{\sqrt{1 - (v_1^2/c^2)}} \quad (1)$$

and

$$\nu_2' = \nu \frac{1 - (v_2/c) \cos \alpha_2}{\sqrt{1 - (v_2^2/c^2)}} \quad (2)$$

These are the relativistic Doppler equations applied to the two receivers. Equation (2) can, however, be recast. The vector \mathbf{v}_2 is the sum of \mathbf{v}_1 and \mathbf{V} ; \mathbf{v}_1 is, however, in the source frame of reference and \mathbf{V} is in the frame of reference of receiver 1 so that it is necessary to resort to the relativistic summation of velocities. The cartesian components of \mathbf{v}_2 are, therefore:

$$\left. \begin{aligned} v_{2x} &= \frac{V_x + v_1}{1 + (V_x v_1/c^2)} \\ v_{2y} &= \frac{\sqrt{1 - (v_1^2/c^2)}}{1 + (V_x v_1/c^2)} V_y \\ v_{2z} &= \frac{\sqrt{1 - (v_1^2/c^2)}}{1 + (V_x v_1/c^2)} V_z \end{aligned} \right\} \quad (3)$$

α_2 is the angle between \mathbf{v}_2 , whose direction cosines are v_{2x}/v_2 , v_{2y}/v_2 , v_{2z}/v_2 , and \mathbf{l} , whose direction cosines are $\cos \alpha_1$, $\sin \alpha_1$, and 0. Therefore:

$$v_2 \cos \alpha_2 = v_{2x} \cos \alpha_1 + v_{2y} \sin \alpha_1 \quad (4)$$

The denominator of equation (2) is $\sqrt{1 - (v_2^2/c^2)}$, where $v_2^2 = v_{2x}^2 + v_{2y}^2 + v_{2z}^2$.

Using equation 3:

$$\sqrt{1 - (v_2^2/c^2)} = \frac{1}{1 + (V_x v_1/c^2)} \sqrt{\{1 - (V^2/c^2)\}[1 - (v_1^2/c^2)]} \quad (5)$$

Using equations (1), (3), (4), and (5) in (2):

$$\nu_2' = \frac{\nu_1'}{\sqrt{1 - (V^2/c^2)}} \times \left[1 - \frac{\{(V_x/c)(\cos \alpha_1 - (v_1/c)) + (V_y/c)\sqrt{1 - (v_1^2/c^2)}\} \sin \alpha_1}{1 - (v_1/c) \cos \alpha_1} \right] \quad (6)$$

From Pauli²:

$$\frac{\cos \alpha_1 - (v_1/c)}{1 - (v_1/c) \cos \alpha_1} = \cos \alpha_1'$$

and

$$\frac{\sqrt{1 - (v_1^2/c^2)} \sin \alpha_1}{1 - (v_1/c) \cos \alpha_1} = \sin \alpha_1'$$

Therefore:

$$\nu_2' = \frac{\nu_1'}{\sqrt{1 - (V^2/c^2)}} \left[1 - \frac{V}{c} \left(\frac{V_x}{V} \cos \alpha_1' + \frac{V_y}{V} \sin \alpha_1' \right) \right] \quad (7)$$

$(V_x/V) \cos \alpha_1' + (V_y/V) \sin \alpha_1'$ is $\mathbf{V} \cdot \mathbf{l}'$. Therefore:

$$\nu_2' = \frac{\nu_1'}{\sqrt{1 - (V^2/c^2)}} \left[1 - \frac{V}{c} \cos \phi_1' \right] \quad (8)$$

Equation (8) is the required equation. It expresses the frequency received at one receiver in terms of the frequency received at the other. It contains only observables. ϕ_1' is the angle between the source direction measured from receiver 1, and the velocity of receiver 2 relative to receiver 1. The term

$(V/c) \cos \phi_1'$ is similar to the classical Doppler term, but the angle ϕ_1' involves the source direction as seen from receiver 1 and so includes diurnal aberration. If a geocentric coordinate system had been chosen, the Doppler term could not have included diurnal aberration. $1/\sqrt{1-(V^2/c^2)}$ is a time-dilatation factor, and the right hand side of equation (8) is the product of a time-dilatation and a Doppler shift factor; all of the terms must be defined correctly.

It makes no difference which receiver is labelled number 1, and, therefore, equation (8) can equally well be written

$$v_1' = \frac{v_2'}{\sqrt{1-(V^2/c^2)}} \left[1 - \frac{V}{c} \cos \phi_2' \right] \quad (9)$$

From equations (8) and (9):

$$\cos \phi_2' = - \frac{(\cos \phi_1' - (V/c))}{1 - (V/c) \cos \phi_1'} \quad (10)$$

Equations (8) and (10) have the same form as equations (15) and (16) of Pauli². These are the relativistic Doppler equation and the equation for transforming angles from the source frame to the receiver frame of reference. In the simple Doppler case involving one source and one receiver, however, the velocity involved is the relative velocity between source and receiver, whereas in my equations (8) and (10), the velocity involved is the velocity of one receiver relative to the other one. The velocity of the source relative to the receivers, which is unobservable by means of an interferometer, is not involved.

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Mode of propagation of penumbral waves

THE solar atmosphere provides an interesting possibility for the study of low frequency waves both in magnetic and non-magnetic regions, and the most spectacular result (see ref. 1) has been the discovery of waves propagating outwards over sun-spot penumbrae with periods of 180–240 s, horizontal wavelengths of 2,350–3,800 km, and horizontal phase velocities of 12–25 km s⁻¹. The theoretical interpretation of these waves has aroused considerable interest and, in particular, a number of authors have attempted to identify the precise mode of wave propagation involved. The modes proposed include Alfvén waves¹, acoustic waves², Lamb waves³, and magnetogravity waves of the 'plus' type vertically trapped at photospheric levels⁴. In the face of so many possibilities, further progress will probably depend on a more adequate observational knowledge of the actual propagation characteristics of the penumbral waves. Of special importance would be a determination of the direction of the particle velocity with respect to the wave propagation vector.

I have therefore explored the conditions of observability as a function of heliocentric angle for purely longitudinal (that is, particle velocity parallel to the propagation vector) and purely transverse (that is, normal to the propagation vector) waves and I present here observations that tend to support a predominantly longitudinal mode of wave propagation.

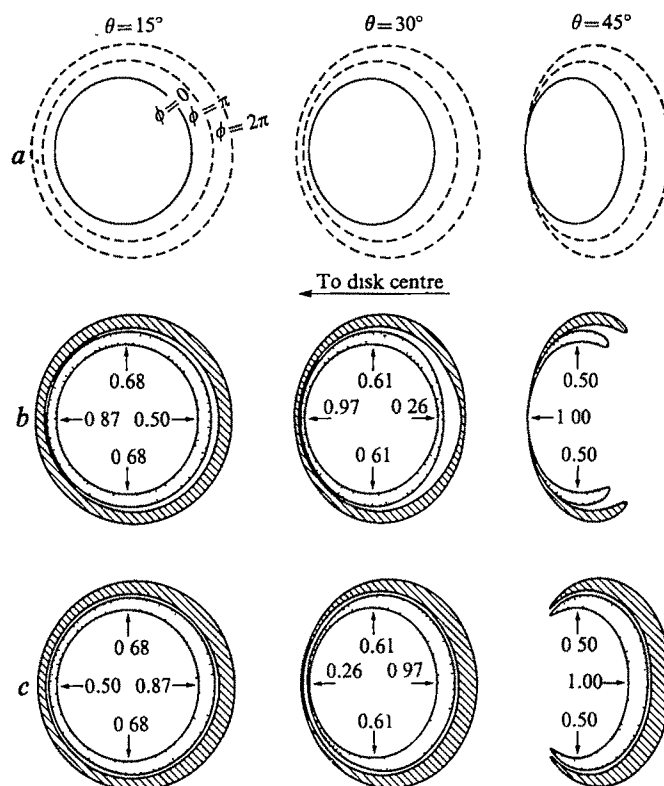


Fig. 1 *a*, Loci of positions with phase $\phi = 0, \pi$, and 2π in a hypothetical wave pattern (see text) for different values of the heliocentric angle θ . The wave is assumed to propagate at an angle $\alpha = \pi/4$ with respect to the vertical. Regions with line-of-sight velocity $|v_{||}| > 0.25v_0$, where v_0 is the amplitude of the wave, are shown for longitudinal waves (*b*), and transverse waves (*c*). The maximum line-of-sight velocity (in units of v_0) is given for certain position angles. Shaded and hatched regions refer to receding and approaching velocities, respectively.

Consider a hypothetical, progressive wave symmetrical with respect to the spot axis, such that at a fixed instant of time the horizontal distance from the centre of the spot is equal to r for positions in the wave with phase $\phi = 0$ and $r + \lambda \sin \alpha$ for $\phi = 2\pi$. Here, λ is the wavelength of the wave, assumed to propagate at an angle α with respect to the vertical. Projected on to a plane perpendicular to the line of sight, the loci of positions in the wave with $\phi = 0$ and 2π represent ellipses. The $\phi = 2\pi$ ellipse is displaced by an amount $\lambda \cos \alpha \sin \theta$ towards the solar limb with respect to the $\phi = 0$ ellipse. Positions in the wave with $\phi = 0, \pi$, and 2π are shown in Fig. 1*a* for different values of the heliocentric angle θ , assuming $\alpha = \pi/4$, $r = 6,500$ km, and $\lambda \sin \alpha = 3,000$ km. As the wave propagation vector is observed to have a horizontal component, a value of α close to zero cannot account for the observations, but a determination of α is not available at present.

Depending on the type of wave involved, variations in the line intensity may⁵ or may not⁶ occur. The line intensity is not a simple function of the particle velocity in the wave. But provided that the wave amplitude is independent of position angle in the spot, positions with the same phase will also show the same line intensity. The appearance of an 'intensity' wave at different heliocentric angles can, therefore, be visualised from Fig. 1*a*, which shows lines of equal phase.

For a longitudinal wave the particle velocity, v , has the components $v \sin \alpha$ and $v \cos \alpha$ in the horizontal and the vertical directions, respectively, whereas for a transverse wave the corresponding components are $-v \cos \alpha$ and $v \sin \alpha$. Assuming that the velocity amplitude v_0 is constant across the region $\phi = 0-2\pi$, we have calculated the line-of-sight velocity $v_{||}$ of the particles in the wave using the equations given by Maltby⁷. The results are given in Fig. 1*b* and *c* which shows the regions

where $|v_{||}|$ exceeds $0.25v_0$ for longitudinal and transverse waves, respectively.

The direction of particle motion in the waves can be studied by comparing observations of spots situated close to the limb with those of spots near the disk centre. Observations of sunspots at large heliocentric angles are, however, hampered because of foreshortening. On the other hand, Fig. 1*b* and *c* indicates that significant differences in the observability of longitudinal and transverse waves still exist for spots situated at intermediate heliocentric angles. The observability of the waves evidently depends on the direction of wave propagation (that is, on the value of α). If $\pi/4 < \alpha < \pi/2$, transverse waves are observable more easily than longitudinal waves in spots situated near the disk centre.

We have compared hypothetical wave patterns with actual wave patterns observed in spots photographed at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Solar Observatory, Culgoora, between 1971 and 1974. No asymmetry was detected in six spots situated relatively close to the centre of the solar disk ($\theta < 23^\circ$). For the spots observed on November 3, 1971 (ref. 1: Fig. 1) and July 3, 1973 (ref. 8: Figs 1–4) penumbral waves are practically absent on the limb side of the spot, but prominent in other places; these spots have heliocentric angles of 35° and 34° , respectively. The observed asymmetries therefore tend to support an interpretation in terms of a predominantly longitudinal mode of wave propagation. The available data are, however, very limited and more observations will be required before any firm conclusion can be reached.

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Basal complex of Fuerteventura (Canary Islands) is an oceanic intrusive complex with rift-system affinities

THE exposed portions of the Canary Islands largely comprise Cainozoic volcanic rocks erupted sub-aerially in successions essentially specific to each island, though with somewhat complex interrelationships¹. On three islands, Fuerteventura, La Palma, and Gomera these rocks rest unconformably on older formations which are similar enough to suggest that a common basement may exist. The largest area of the basement complex rocks is the Betancuria Massif^{2–4}, Fuerteventura. Gastesi⁴ has suggested that similarities exist between the Betancuria Massif and other ophiolite complexes (such as the Troodos Massif of Cyprus^{5,6}) generally held to be representative of oceanic crust formed at certain constructive plate margins.

The most obvious feature of the complex is the striking dyke swarm in which the dyke intensity sometimes reaches 100%. The oldest rocks seen at the present erosion level form the host rock to the dyke swarm and comprise basic and alkaline rocks of plutonic aspect together with marine sedimentary rocks intercalated with submarine volcanics (Table 1*a*). The sediments comprise laminated shales and

Table 1 Revised succession* from the Betancuria Massif

	Unconformity
	<i>d</i> Late syenitic and trachytic ring complexes Alkali gabbros sometimes related to the ring complexes
Basal Complex	<i>c</i> Basic and ultrabasic intrusions, with associated dykes
	<i>b</i> Main phase dyke swarm
	<i>a</i> Basic and alkaline rocks of plutonic aspect Mesozoic and Tertiary sediments with submarine volcanics

*Date from a reinvestigation conducted during 1974, and recent work by the Instituto Lucas Mallada.

siltstones, turbiditic sandstones and thin tuffs and limestones; Cretaceous foraminifera have also been found⁷. Weak regional metamorphism and metasomatism have affected all the strata; in addition, the sediments close to the ultrabasic and basic intrusions (Table 1*c*) have been thermally metamorphosed. Deformation has imposed a regional cleavage and much of the observed sequence is inverted by large scale, WNW–ESE trending folds. According to Rothe⁷ these rocks are similar to rocks from the African continent and from Maio in the Cape Verde Islands. At the top of the sedimentary sequence interbedded hyaloclastites and pillow lavas appear, leading up into a thick series of submarine volcanics in which both basaltic and trachytic types are represented. The bedded volcanic rocks are traversed by synchronous sheet intrusions of similar petrography to the pillow lavas.

Because of the subsequent intrusion of a dyke swarm it is difficult to trace stratigraphic continuity. It seems, however, that tuffs become much more common passing upwards: calcareous tuffs appear with thin limestones which become more frequent until thick fragmental limestones containing abundant shell debris are seen. Fossils of Miocene to Eocene age have been recorded from this horizon⁸. It seems that these sediments are of shallow water origin, and according to earlier work² the sequence continues upwards into subaerial tuffs and agglomerates. In the reinvestigated area the succession is terminated by an unconformity above which the Basalt Series was erupted.

In the south-western part of the Betancuria Massif, the host rocks to the dyke swarm comprise basic and alkaline rock of plutonic aspect. The relationship between these rocks and the Mesozoic bedded rocks is not clear as the contact is obscured by the younger dyke swarm. Intruding both the plutonic and the bedded rocks are abundant veins and occasional small intrusive bodies of syenite and small carbonatite dykes which occupy a zone marginal to the plutonic rocks and which do not extend far into the sediments and submarine volcanics.

All of those rocks are traversed by a swarm of dominantly basaltic and ankaramitic dykes with a general NNE–SSW trend (Table 1*b*). The swarm contains dykes of several ages, but the majority belong to a major phase of permissive emplacement which involved no deformation of the host rock other than distension of the crust. Throughout this phase of emplacement later dykes injected earlier ones and, consequently, individual dykes may be dismembered, with the fragments widely separated. Both the host rocks and dykes of this phase show the imprint of a greenschist facies metamorphism and, as in the oceanic greenstones, are typically devoid of a pervasive foliation⁹. The recognition of thermal aureoles around basic and ultrabasic bodies

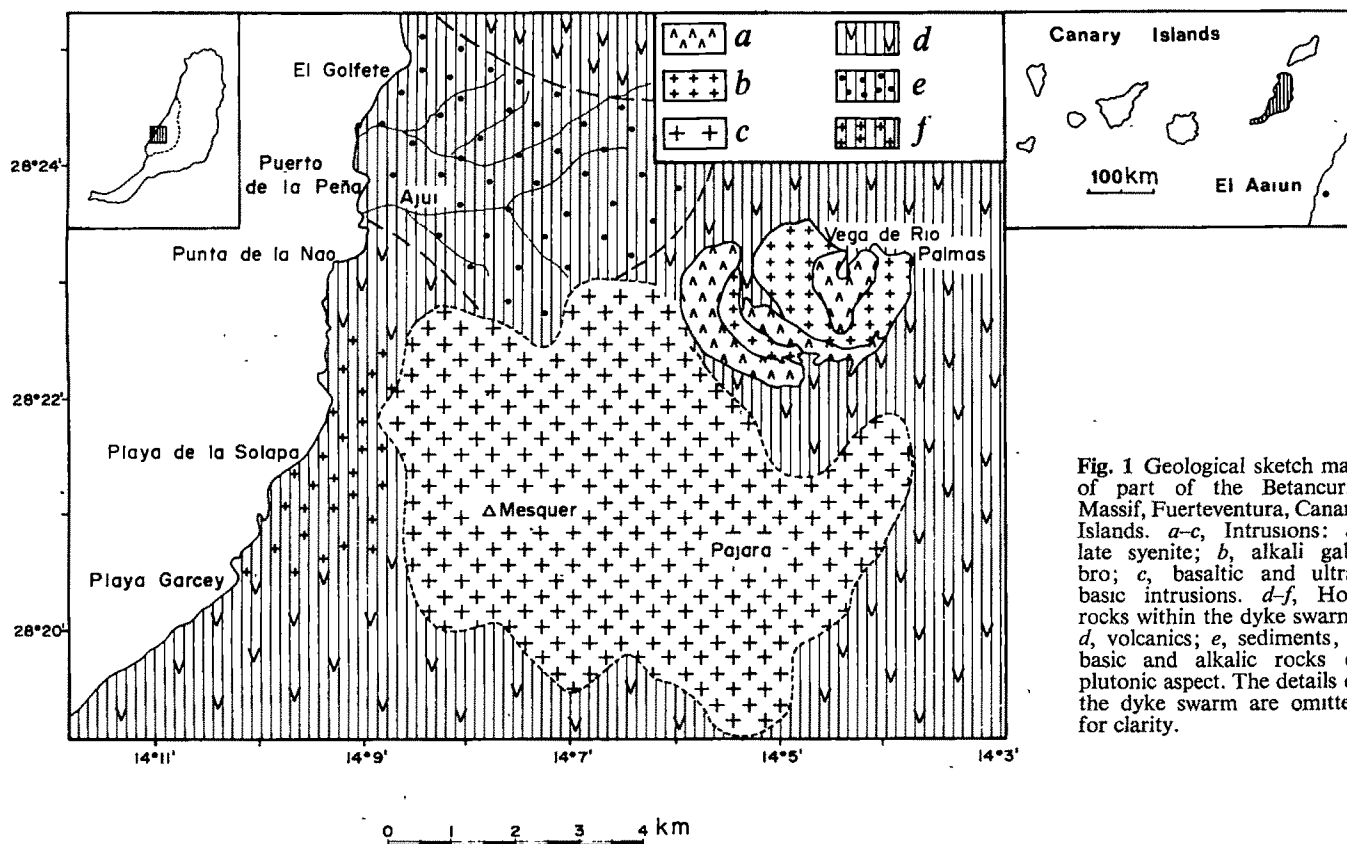


Fig. 1 Geological sketch map of part of the Betancuria Massif, Fuerteventura, Canary Islands. *a-c*, Intrusions: *a*, late syenite; *b*, alkali gabbro; *c*, basaltic and ultrabasic intrusions. *d-f*, Host rocks within the dyke swarm: *d*, volcanics; *e*, sediments, *f*, basic and alkalic rocks of plutonic aspect. The details of the dyke swarm are omitted for clarity.

(Table 1c) which intrude the earlier parts of the complex has made possible the distinction of at least two periods of dyke intrusion in the main phase. The proportion of dykes to host rock increases markedly towards these younger intrusives, implying that during emplacement basaltic magmas from the intruding body was fed actively into fractures within the overlying crust and that it was into that heavily dyked host that the pluton was finally emplaced. The older dykes (whose parent pluton is not exposed at the present erosion level) and those associated with the more recent activity are petrologically similar, are affected by the same greenschist facies metamorphism, and strike in the same direction. The field evidence thus suggests repeated plutonic and hypabyssal intrusion in the same environments within a single extensional stress field.

Subsequent to the relaxation of this stress field, sheet intrusions associated with still later syenitic and trachytic ring complexes were emplaced into the dyke swarm. In addition, younger, zeolite facies basaltic dykes of unknown affinities also occur.

Most of the later dykes follow the same trend as the earlier ones where the dyke intensity approaches 100% though the concordance is much less noticeable away from this zone. Apparently, the initial trend was dictated by regional tensional stresses, but subsequent emplacement was controlled more by the grain imposed on the crust by the dyke swarm. Some of the ultrabasic and gabbroic intrusions are parallel to the orientation of the dyke swarm, others have been emplaced by passive stoping, sometimes associated with ring fracturing. The latter are succeeded by syenite and trachyte intrusions which form large partial ring dykes and sheet intrusions such as those at Vega de Río Palmas.

Although the individual components of the Basal Complex, are similar to those forming ophiolites^{4,10}, the relationships which define an ophiolitic terrain¹⁰ are not seen, and it is, therefore, uncertain whether the Basal Complex represents a fragment of normal oceanic crust. There can be little doubt that the bedded rocks were erupted and de-

posited on an ocean floor; but the problem remains. was this part of a marginal basin underlain by continental crust or was it part of the new Atlantic crust? The bedded rocks range in age from the Upper Cretaceous⁷ to the Mid-Tertiary⁸, though that range may be more extensive—Rothe⁷, for example, has suggested that the older sediments may be Jurassic, and that the Mesozoic rocks at least have been involved in Alpine-style tectonics, and are, therefore, presumably related to the Atlas tectonism on the African continent. Further evidence that the sediments form part of a continental margin basin is provided by seismic profiles from the area between Fuerteventura and the mainland, which indicate the presence of some 10 km of sediment¹¹. There is, however, a divergence of opinion as to the nature of the crust beneath these sediments^{2,11-14}. Further, although seismic data confirm the existence of an important fracture west of Fuerteventura^{13,14} there seems to be little in the available petrological and geochemical data to suggest that the islands in the west and east of the archipelago are emplaced over significantly different crust. Data from the basal complexes do not indicate much difference in the early stages, and studies^{15,16} of Cainozoic magmas reveal inter-island relationships which are complex, and do not suggest any particular relationship between geochemistry and distance from the African continent.

Radiometric data suggest that the majority of the Fuerteventura Basal Complex intrusives were emplaced in mid-Tertiary times. Ages from dykes of the main phase range from 32 Myr to 46 Myr; the age of the older basic and alkalic rocks of plutonic aspect is not yet known, and the only pluton which has so far been dated (20 Myr) is one of the youngest: the outer ring syenite of the Vega de Río Palmas Complex (ref. 17, and D. Rex and I. G. Gass, personal communication). Basal Complex activity must have terminated shortly after 20 Myr BP, as the beginning of subaerial volcanicity has been dated at >16.5 Myr (ref. 17).

The tensional conditions which permitted the intrusive phase on Fuerteventura may well represent mid-Tertiary

rifting and may indicate the existence of an embryonic spreading axis which aborted after a relatively short time, a view already postulated by Gastesi⁴. The extensional stress field had probably relaxed by the time of emplacement of the syenite ring complexes approximately 20 Myr BP. The variations in trend of the Basal Complex dyke swarms on La Palma, Gomera and Fuerteventura may indicate several 'spreading' attempts but until more precise chronological correlation is possible, that remains speculative. The case for a propagating fracture model to explain the continuing volcanicity of the Canary Islands has been propounded¹, and the proposed model for mid-Tertiary rifting does not conflict with that idea.

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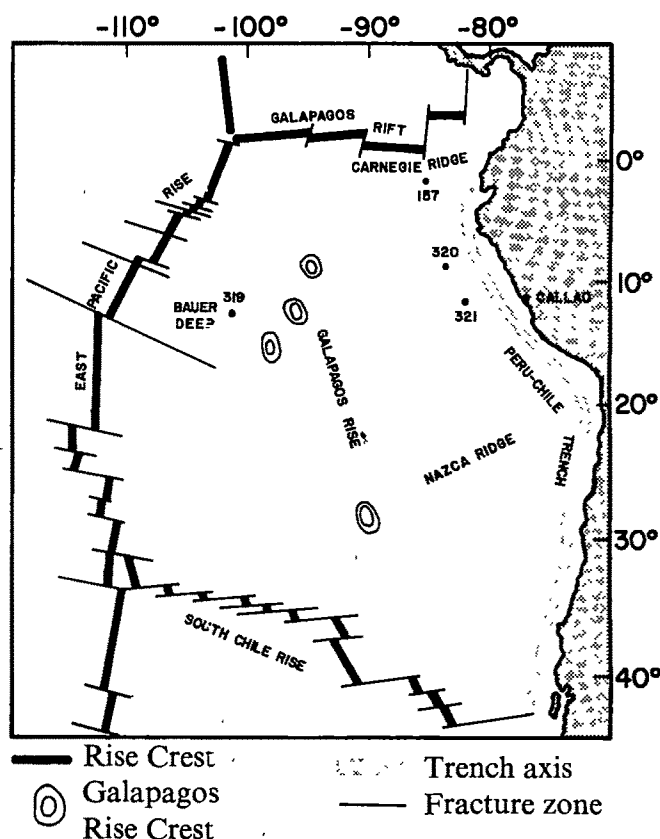


Fig. 1 The Nazca Plate showing DSDP sites 319, 320, 321 and 157.

that the determination of the ages of sites 319 and 320 with respect to the magnetic reversal time scale will be precluded. Table 1 shows the basement ages for the sites, estimated from the overlying sediments.

The total penetration of basement rocks at three sites was 105 m, of which only 15 m were recovered for study. A total of 35 basalt and 32 sediment samples (vertically oriented) was taken from the leg 34 drill sites. In addition, we have studied four samples from site 157 of the DSDP leg 16, also on the Nazca Plate.

Recovery of coarse grained massive basalts at several sites, in addition to pillow sequences, justifies the previous suggestion⁴ that dredged material from the upper surface of oceanic layer 2 is unrepresentative of the layer as a whole. Coring in the massive flow units produced good recovery at sites 319 and 321, and pillow material resulted in poor recovery at all sites. Table 1 shows the average natural remanent magnetisation (NRM) intensity for each site, weighted for the differential recovery of fine and coarse material. Pillow material was significantly less intensely magnetised than the coarse grained flow interiors, and this is shown by comparison of the average magnetisation at sites 321 and 157, which were dominated by

Magnetic results from basalts and sediments from the Nazca Plate

THE drilling sites for leg 34 of the Deep Sea Drilling Project (DSDP) were in the Nazca Plate (Fig. 1), which was formed by the spreading from two ridges, the fossil Galapagos Rise and the currently active East Pacific Rise. Spreading half rates on the East Pacific Rise vary from 8 to 10 cm⁻¹ (refs 1-3) and are the highest on any presently active ridge. Fast spreading is associated with subducted basement topography, a situation that enables structural provinces within the Nazca Plate to be clearly delineated. The near equatorial latitudes of the sites with poor magnetic anomaly development mean, however,

Table 1 Basement ages and average intensity of undemagnetised natural remanent magnetisation for the basement basalts

Site	Estimated age of sediment directly above basement (Myr)	Site average NRM intensity (e.m.u. cm ⁻³ × 10 ⁻⁴)	
		Averaged over all NRM values	Weighted for differential recovery of fine and coarse grained basalts
157	8	138.0 ± 24.0	—
319	14-15	23.8 ± 5.7	17.4 ± 5.9
320	26-30	13.2 ± 2.9	13.2 ± 7.6
321	39-40	103.0 ± 23.0	96.0 ± 23.0

Table 2 Mean site inclinations and equivalent palaeolatitudes for sediments and underlying basement basalts

Site	319	320	321	157
Site latitude	13°S	9°S	12°S	2°S
Equivalent dipole inclination	25°	18°	23°	4°
Basalt average inclination	+54±4°	-25±9°	-20±2°	-31±4°
Equivalent dipole palaeolatitude (north or south)	35±4°	13±5°	10±1°	17±3°
Sediment average inclination				
(a) Assuming all samples have same polarity	14±7°	8±10°	11±4°	
Equivalent dipole palaeolatitude (north or south)	7±4°	4±6°	6±2°	
(b) Assuming polarity indicated by sign of inclination	16±6°	31±6°	15±3°	
Equivalent dipole palaeolatitude (north or south)	8±3°	17±4°	8±2°	

coarse grained material, and site 320, which was exclusively oxidised pillow basalts. Site 319 is a 60-m basement section, and the remanence is dominated by a 10–20-m section of coarse grained flow material.

For the basalt samples, the ratio of remanent intensity to induced magnetisation, Q , was in all cases greater than 1. The range of Q was large, for massive flows varying from 2 to 28 and for fine grained basalts varying from 2 to 56. It would seem that for these samples, the remanent intensity will be dominant.

The coarse grained basalts of hole 319A had very large, soft components of magnetisation that were directed vertically upwards. This hole was drilled with a magnetic steel drilling assembly, a configuration that is sometimes responsible for a drilling remanent magnetisation. Lowrie and Kent⁵ have shown that for some of the leg 34 coarse grained samples, exposure to a field as small as 4 oersted for only a few hours, a time sufficient to drill 9.5 m of basalt, can generate the observed soft components. The massive flow basalts of site 321, which were drilled with a non-magnetic monel metal drilling assembly, had soft secondary components of magnetisation which were directed downwards. At all of the leg 34 sites, the geomagnetic field, at present, is inclined upwards at a shallow angle. The soft components of NRM measured in the laboratory seem to be some combination of drilling and viscous remanent magnetisation (VRM) acquired on the ocean floor. Lowrie and Kent⁵ have also shown that these coarse grained samples can acquire a VRM component that is comparable in intensity with the stable component in less than 7×10^5 yr. If this is the case, then *in situ* VRM will be the dominant remanence and should mask or distort any stable thermoremanent magnetisation (TRM). In areas of the oceanic crust which are predominately coarse grained basalts, the magnetic anomaly which is evident at the sea surface will be composed of some combination of a viscous remanence, the induced magnetisation (both in the direction of the present field), and the original thermoremanence. In these areas, the magnetic anomalies will tend to be positive, and the variations in the anomaly pattern will be unlikely to correspond to a magnetic reversal time scale.

Table 2 shows the inclinations for the partially demagnetised basalt samples, averaged for each site. The values for sites 320 and 321, the oldest sites, are close to those expected for an axial

centred dipole at the latitude of each site. Sites 157 and 319, however, show significant departures from the inclinations expected at the present-day latitudes of the sites. Interpretation of these inclinations as actual palaeolatitudes would require unusually large plate velocities of 20 cm yr⁻¹ and would also require that the Nazca Plate moved rapidly southward between 26 and 15 Myr ago, and then northward to the present latitude between 15 Myr ago and the present.

A more reasonable explanation is that the basalts taken at sites 157 and 319 were erupted over a time interval that was too short to average over a quasi-period (10^3 yr) of secular variation. A possible, but less likely explanation is that during extrusion, the geomagnetic field was undergoing a polarity transition or some systematic deviation. Tectonic movement of crustal blocks, although present to a small extent, is an unlikely cause of the rotation of the magnetic directions. Chemical remagnetisation due to oxidation can be eliminated since similar directions are indicated for both the oxidised and unoxidised samples. The tight and consistent grouping of the inclinations of site 319 indicates that a short period of extrusion is the likely explanation.

Additional information about the palaeolatitude of the Nazca Plate is available from the sediments which overlie the basement basalts. Since the sedimentation rate in this area has been estimated to be 2.8–4.2 cm every thousand years⁶, each sediment sample with a diameter of 1.9 cm covers about 450–680 yr. At each site, the sediment column directly above the basement was cored over a few tens of metres, a thickness representing about 10^6 yr. When the sampling interval is extended over this longer time period, the sediment inclinations in Table 2 show a southward palaeolatitude change of 3–5° for the Nazca Plate during the past 15 Myr and no latitude change between 15 and 40 Myr BP. This agrees with previous studies^{1–3,6} that the motion of the plate has been largely in an eastward direction.

The study of the palaeomagnetism of oceanic basement rocks can provide information regarding a rough minimum of the amount of time involved in crustal genesis. The presence of several magnetic reversals in a column of basalt flows implies a time of formation of 10^5 – 10^6 yr or greater, whereas a tight grouping of inclinations of a single polarity (that deviate

Table 3 Comparison of rock magnetic parameters for site 319

Type of material	Mean particle size (μm)	Curie temperature T_c (°C)	Median demagnetising field MDF (oersted)	Weak field susceptibility k (e.m.u. cm ⁻³ × 10 ⁻⁴)	Intensity of NRM J_0 (e.m.u. cm ⁻³ × 10 ⁻⁴)	Strong field saturation moment J_s (e.m.u. cm ⁻³)
Oxidised pillows or flow margins (5)*	4.9	340	258	0.86	8.6	0.50
Oxidised coarse grained massive flow interiors (4)	20.0	290	167	1.26	12.0	0.97
Unoxidised coarse grained massive flow interiors (5)	19.0	147	64	8.4	65.0	1.4

Samples were grouped into one of three categories for comparison of parameter variation with low temperature oxidation state and grain size

*Numbers in parentheses are number of samples

significantly from the expected inclination) may imply extrusion within 10^2 yr. The inclination data from site 319 suggest that the basement crust sampled at this site was formed in a relatively short time. Too few oriented samples were recovered from site 320 for any interpretation to be made. The inclinations from site 321 were tightly grouped with the exception of one reversed inclination from a sample at the very top of the basement section. Re-examination of the drill core showed that this section had been inverted during drilling. If this reversal is the result of coring, then the column of basement basalts at this site would also have been generated over a short ($\leq 10^2$ yr) time period. At site 157 the three top samples were normally magnetised and overlie a reversely magnetised sample. Re-examination of the drill core showed no evidence that this reversal was the result of drilling. If this bottom sample represents a true field reversal, then this column of basement basalts is recording a period in which the geomagnetic field is reversing or the time of formation of this thin layer of ocean crust is greater than 10^5 – 10^6 yr.

The basalt samples were divided into three categories, based on the size of the titanomagnetite grains present and the degree of alteration of these grains by low temperature oxidation.

(1) The first category consists of the unoxidised interiors of massive volcanic units characterised by large unoxidised titanomagnetite grains, 20–30 μm in mean size with grains up to 100 μm .

(2) Second, there are samples similar to the first, but now oxidised to titanomagmaemite and physically subdivided by cracks due to alteration.

(3) Lastly, there are fine grain pillow basalts with skeletal grains of titanomagmaemite, 2–5 μm in size. No unoxidised pillow material was recovered.

Division of the basalt samples into these categories makes possible the correlation of the magnetic parameters with low temperature oxidation state and grain size. Data from Site 319, which contained all three types of basalt samples, enable comparisons to be made without a wide variation in age or geographic location. Table 3 shows the magnetic properties for these groups of samples. The unoxidised massive flows are more intensely magnetised than either the oxidised massive flows or the oxidised pillow basalts. Similar to what has been reported from laboratory studies⁷, the natural low temperature oxidation of titanomagnetite to titanomagmaemite causes a decrease in intensity of remanence and saturation moment, and an increase in coercivity and Curie temperature. Weak field susceptibility also decreases with oxidation.

Low temperature oxidation is important in the control of the magnetic properties of submarine basalts, particularly in the fine grained pillow material. Evidence for hydrothermal alteration at moderate temperatures was absent from all four sites on the Nazca Plate. The presence of unoxidised titanomagnetite grains in the massive volcanic units of the 40-Myr-old site 321 and 15-Myr-old site 319 indicates that flow structure can be a more important factor in the alteration history than age alone.

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Evidence for a new aluminium phosphate phase from reaction rate of phosphate with aluminium hydroxide

THE reaction between phosphate and aluminium hydroxide is important in soil fertility and also in the management of systems whereby waste water is disposed directly on to the land. The retention of phosphate by a sandy topsoil during 50 yr of application of raw sewage water cannot be accounted for by adsorption on soil constituents alone¹. It was suggested that precipitation of the phosphate had probably taken place. Later investigations on this soil (Beek, personal communications) indicated the presence of an aluminium phosphate as the main fraction of phosphate retained. We report here results for the production of aluminium phosphate which may have a bearing on waste water management.

Most of the older research on the precipitation of aluminium phosphate involved systems using high phosphate concentrations and/or high temperature^{2–5}. The experiments reported here were done at 20.5 ± 0.1 °C with an initial phosphate concentration of $0.36 \text{ mmol dm}^{-3}$ at pH 5. X-ray amorphous aluminium hydroxide with a specific surface area (B.E.T.) of $226 \text{ m}^2 \text{ g}^{-1}$ was used⁶. The pH was kept constant (± 0.1) by the addition of HCl. From aliquots of the suspensions the phosphate concentration was measured after filtration through a Millipore (0.1 μm) filter.

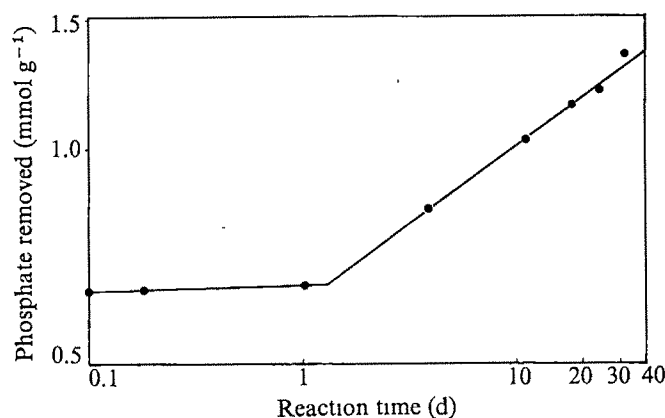


Fig. 1 A semi log plot of the measured phosphate concentration as a function of reaction time for a suspension concentration of $0.2 \text{ g dm}^{-3} \text{ Al(OH)}_3$. For the period 1–40 d, the first order rate constant, $K_{1-40 \text{ d}}$ equals 0.0069 d^{-1} .

The results of the measurements are given in Figs 1 and 2. Figure 1 shows that, for the time period from ~1 to 40 d, the rate can be described by first order kinetics.

$$\ln \frac{c_0'}{c} = Kt \quad (1)$$

where $c_0' = c$ at about 1 d of shaking, K = rate constant d^{-1} , c = phosphate concentration. Similar observations have been reported⁷ for the reaction of phosphate with $\alpha \text{ Al}_2\text{O}_3$ and with kaolinite.

The value of K depends on the amount of specific surface area per litre and on the charge of the surface which is dependent on pH. Another way to express the measured data is in terms of the amount of phosphate removed from the solution per g Al(OH)_3 . This is shown in Fig. 2 using a double log plot.

Figure 2 shows two different straight lines. The first line (up to about 1 d) represents the fast adsorption on the surface of the

$\text{Al}(\text{OH})_3$, a short term reaction which has been shown previously⁸. After 1 d the reaction rate increases markedly compared with the first process, a phenomenon which remains hidden in Fig. 1. Such an increase in reaction rate could conceivably be caused by the initiation of the growth of a crystalline aluminium phosphate. This supposition was tested using electron microscopy, electron diffraction and X-ray diffraction. These techniques were applied after a reaction time of 62 d. Crystalline particles of a platy structure were visible, some of them

From the evidence given here it is not unlikely that the growth of an aluminium phosphate phase is the rate determining step after 1 d in this system. Similar experiments were done with $\alpha\text{-Al}_2\text{O}_3$ and with solutions containing different ions at pH 5 and 6. It is interesting to note that the intersection point of the two straight lines in Fig. 2 occurs at a reaction time of 4 d for the case of $\alpha\text{-Al}_2\text{O}_3$. The results of these experiments which are comparable with the ones given here will be published elsewhere.

Table 1 *d* Spacings of the aluminium phosphate investigated compared with the tabulated values of sterrettite, meta-variscite and variscite

X-ray diffraction	Electron diffraction	Sterrettite*	Tabulated values of		Variscite*	
			Meta-variscite*			
		6.94	6.37 (4)	2.16	5.365 (1)	1.952
		5.25	4.77	2.11	4.82	1.903
4.90 (1)	5.26	4.88 (1)	4.575 (3)	2.07	4.257 (1)	1.852
4.51 (2)	4.55	4.51 (2)	4.43	2.01	3.887	1.809
3.75		3.79 (4)	4.25 (2)	1.961	3.625	1.779
		2.90 (3)	4.04	1.933	3.366	1.752
		2.76 (5)	3.52	1.865	3.204	1.715
2.62	2.63	2.66	3.37	1.824	3.039 (1)	1.656
		2.44	3.25	1.793	2.957	1.640
		2.33	3.12	1.771	2.853	1.604
	2.25	2.24	3.02	1.743	2.63	1.588
		2.07 (6)	2.90	1.721	2.568	1.574
		1.87	2.79	1.670	2.48	1.557
		1.77	2.71 (1)	1.646	2.39	1.519
	1.72	1.71 (7)	2.65	1.630	2.33	1.496
		1.61	2.59	1.612	2.278	1.438
	1.52	1.54	2.50	1.591	2.136	
		1.49	2.41	1.560	2.081	
		1.37	2.30	1.539	2.047	
		1.35	2.21'		2.014	
	1.31	1.31		plus 15 lines to 1.258		

* Sterrettite (ASTM 2.0177); meta-variscite (ASTM 15-311); variscite (ASTM 15-281). Numbers in parentheses refer to intensities.

exhibiting an hexagonal shape, the size ranging from 0.5 to 4 μm .

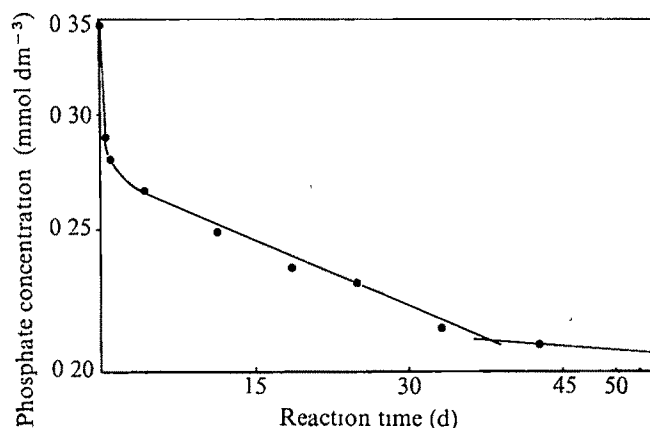
Crystals with the correct thickness all showed electron diffraction patterns of which the *d* spacings were measured using gold pattern as reference. All the crystals examined gave the same diffraction pattern. Table 1 shows these *d* spacings along with those obtained by X-ray diffraction and those of the minerals sterrettite, variscite and meta-variscite.

There is good agreement between the measured values and those of sterrettite. The formation of this compound from solutions containing aluminium and phosphate at pH 5.5 has been reported before⁵.

Fig. 2 A double log plot of the amount of phosphate removed as a function of reaction time. Expressing the lines shown as, $\log X = \log K' + \phi \log t$, with X = phosphate removed (mmol g^{-1}), K' phosphate removed at $t = 1$ d, (ϕ and K' are constants).

$$\log K' = -0.19; \phi = 0.010; t < 1.1 \text{ d.}$$

$$\log K' = -0.20; \phi = 0.230; t > 1.1 \text{ d.}$$



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Excess of atmospheric neon in pumice from the Islands of Lipari

LORD RAYLEIGH¹ reported an excess of neon (over argon) in pumice from the Lipari Islands. Dymond and Hogan² have reported excesses of neon in mid-oceanic tholeiitic basalts and have suggested that they indicate primordial neon. As the search for primordial gases in terrestrial rocks has an important bearing on our understanding of the formation and thermal history of the Earth, we decided to look at the isotopic composition of the neon in pumice from the Lipari Islands. We

Table 1 Helium, neon and argon contents in the Lipari pumice*

Sample	Treatment	^4He ($\times 10^8 \text{ cm}^3 \text{ g}^{-1}$ at STP)	^{20}Ne ($\times 10^8 \text{ cm}^3 \text{ g}^{-1}$ at STP)	$^{20}\text{Ne}/^{22}\text{Ne}$	$^{22}\text{Ne}/^{21}\text{Ne}$	^{36}Ar ($\times 10^8 \text{ cm}^3 \text{ g}^{-1}$ at STP)	$^{36}\text{Ar}/^{38}\text{Ar}$	$^{40}\text{Ar}/^{36}\text{Ar}$	$^{20}\text{Ne}/^{36}\text{Ar}$
A (9.02 g)	Crushing in vacuum	1.22 ± 0.33	9.0 ± 1.1	9.64 ± 0.24	35.4 ± 1.1	0.57 ± 0.06	5.35 ± 0.04	292.6 ± 3.0	15.8 ± 2.0
B (3.22 g)	1st step Crushing in vacuum	19.8 ± 0.31	130 ± 4			20.2 ± 2.0	5.22 ± 0.20	292.5 ± 3.2	6.43 ± 0.90
	2nd step Heating to $\sim 400^\circ\text{C}$	1.6	64.5 ± 6.5	9.80 ± 0.06	35.2 ± 0.1	7.0 ± 0.8	5.30 ± 0.04	293.1 ± 5.4	9.21 ± 0.70
	3rd step Heating to $\sim 1,000^\circ\text{C}$	2.73 ± 0.93	14.0 ± 1.6	9.53 ± 0.29	35.7 ± 1.0	9.6 ± 1.0	5.29 ± 0.04	280.2 ± 1.9	1.46 ± 0.22
	Total (1 + 2 + 3)	22.5 ± 3.6	208 ± 35	9.80 ± 0.06	35.2 ± 0.1	36.6 ± 2.4	5.29 ± 0.04	289.5 ± 3.0	5.65 ± 0.51
C (0.452 g)	Heating to $\sim 1,000^\circ\text{C}$		517 ± 86	9.76 ± 0.14	34.9 ± 0.4	82.9 ± 8.8	5.30 ± 0.06	293.0 ± 2.6	6.23 ± 0.61
Atmosphere				9.80	3.45		5.35	295.5	0.52

*The quoted errors correspond to a 95% confidence limit. They do not include the error of the standard. An error of about 5% has to be added to the given concentrations to obtain the absolute error.

collected fresh specimens from the largest quarry on the islands and extracted the included gases by crushing in a vacuum line and by stepwise heating. The five noble gases were measured in a mass spectrometer.

Large differences were observed in the noble gas contents in three samples (Table 1) A, B and C, indicating heterogeneity in the noble gas distribution, a feature that also seems to be common in basalts. In all of the samples significant excesses of neon were observed, the $^{20}\text{Ne}/^{36}\text{Ar}$ ratio reaching the value 15.8, as compared with the atmospheric value of 0.52. This excess neon has $^{20}\text{Ne}/^{22}\text{Ne}$ and $^{22}\text{Ne}/^{21}\text{Ne}$ values that are indistinguishable from the atmospheric ratios, in the limit of the given analytical errors (Table 1).

whether they are primordial or atmospheric. The mechanism by which the pumice takes up neon upon its formation needs further study (the fact that the helium is less enriched could be a result of subsequent diffusional losses).

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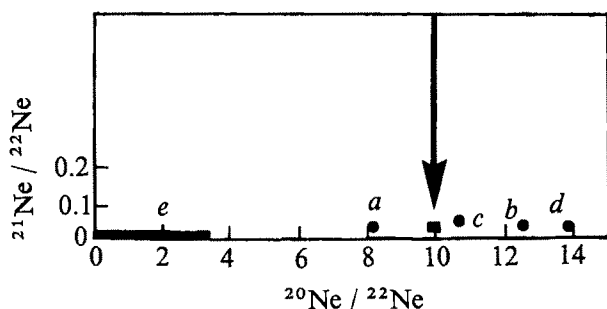


Fig. 1 The isotopic composition of the excess neon in the Lipari pumice compared with known species of primordial neon in extraterrestrial sources (following ref. 3); a, neon A; b, neon B; c, neon C; d, solar wind; e, neon E; arrow, Lipari pumice and atmosphere.

The fact that the same type of neon is observed in crushing and stepwise heating experiments indicates that only one type of neon is present. Further, the observed isotopic composition deviates from any known species of primordial neon in extraterrestrial material (Fig. 1).

These data seem to indicate that the excess neon in pumice from the islands of Lipari is of atmospheric origin. Similar isotopic measurements in the mid-oceanic basalts might clarify

Long range transport of toxaphene insecticide in the atmosphere of the western North Atlantic

OVER the past ten years toxaphene has been used in the USA in larger quantities than any other insecticide, with an estimated consumption of 58 million pounds per year (ref. 1). In spite of this widespread use, the composition of toxaphene remains largely undetermined, and recent work indicates that the technical product contains nearly 200 polychlorinated camphenes². Because of the difficulties in analysing such a complex mixture, little is known about the dissipation of toxaphene in the environment. Residues in soils may persist for years, with volatilisation suggested as a major loss mechanism³. Toxaphene has been identified,

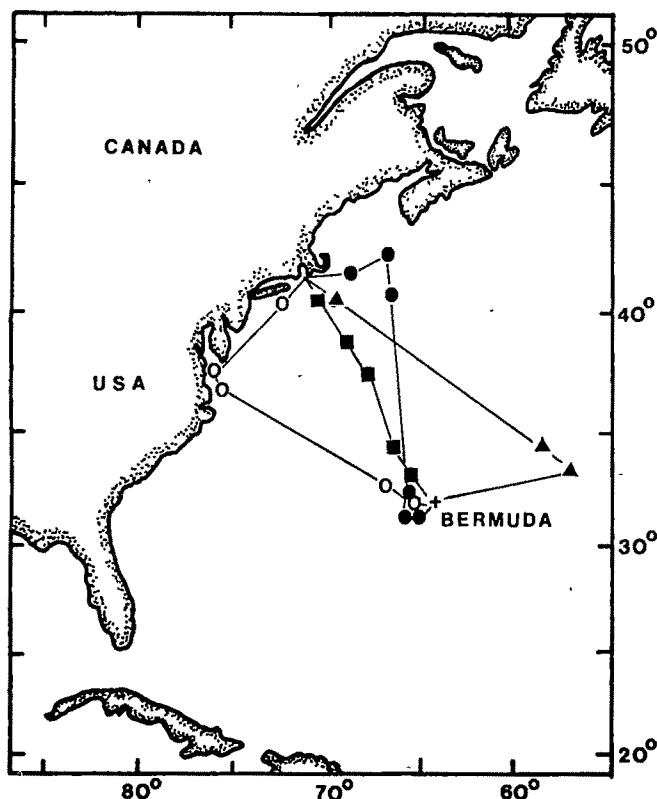


Fig. 1 Atmospheric samples collected from the RV Trident, 1973–74 (see Table 1 for cruise dates). ■, TR-137; ○, TR-145; ●, TR-152; ▲, TR-153.

however, in US air samples in only three locations⁴, all in southern agricultural areas, at levels of 16–2,520 ng m⁻³. The ability of high molecular weight chlorinated hydrocarbons to be transported long distances through the atmosphere has been well established within the past decade. Polychlorinated biphenyls (PCBs)^{5,6}, and the chlorinated pesticides DDT⁷⁻⁹, dieldrin^{7,8}, and chlordane⁹ have been identified in the air over the western North Atlantic. We now report that toxaphene is also carried through the atmosphere at least 1,200 km out to sea.

During 1973–74 we collected 36 air samples from a tower on the south shore of Bermuda and 20 from the bow of the RV Trident on cruises in the western North Atlantic (Fig. 1), and in the spring of 1975 we took six samples at Sapelo Island, Georgia—two on the island and four from the RV Kit Jones, 6–20 km from the coast. The collection system in Bermuda was controlled by wind direction, which enabled us to sample air blowing from a desired sector¹¹. Of the 36 Bermuda samples, 26 were taken only when the wind blew from inside a 90°E–240°SW selected sector (ISS). This air passed over hundreds of kilometres of open ocean and not over the island of Bermuda. Seven samples were collected when the wind blew from outside this sector (OSS) and thus over portions of the island, and the remainder were collected during periods of variable winds.

The samples were taken by drawing 200–3,000 m³ of air through a Gelman A glass fibre filter and then through plugs of polyurethane foam^{6,10}. In most cases we analysed only the foam plugs, since previous work indicated that other chlorinated hydrocarbons are poorly retained by glass fibre filters when large air volumes are sampled. We checked this for a few samples, and found that less than 5% of the total toxaphene was retained by the glass fibre filters, the bulk of the pesticide being found on the foam plugs.

Toxaphene was identified by electron capture gas chromatography on both 1.5% OV-17–1.95% QF-1 and 4%

SE-30–6% QF-1 columns after separation from PCB, DDT, and chlordane by silicic acid column chromatography. The 'toxaphene fraction' from the silicic acid column (3 g, 3.3% water), eluted with dichloromethane after eluting the PCB and DDT–chlordane fractions with petroleum ether, contained the components contributing 60–70% of the total toxaphene electron capture response before separation. Gas chromatograms of the sample extracts and a toxaphene standard contained a number of peaks reminiscent of a PCB pattern (Fig. 2a and b) which were unchanged by treatment of the sample or standard with a mixture of concentrated sulphuric and fuming nitric acids, a procedure which removes residues of DDT, dieldrin, and organophosphate pesticides^{12,13}. The presence of toxaphene was further confirmed by refluxing the sample extracts with alcoholic potassium hydroxide and comparing the resulting gas chromatograms with those of authentic dehydrochlorinated

Table 1 Concentrations of airborne toxaphene

Location*	Dates	Toxaphene† (ng m ⁻³)
Bermuda 32°15'N 64°50'W	Mar.–Dec., 1973	< 0.02–3.3, 1.0 (11)‡
Bermuda 32°15'N 64°50'W	Mar.–Oct., 1974	0.10–1.9, 0.57 (25)‡
Sapelo Island, Georgia 31°15'N 81°05'W	May 20–26, 1975	1.7–5.2, 2.8 (6)‡
Cruise TR-137 33°20'N, 65°15'W 34°40'N, 66°15'W 37°40'N, 68°10'W 38°50'N, 69°15'W 40°30'N, 70°20'W	June 4–9, 1973	0.70 1.2 1.1 0.52 1.6
Cruise TR-145 32°00'N, 65°00'W 32°40'N, 67°20'W 36°40'N, 75°20'W 37°00'N, 76°00'W 37°20'N, 76°10'W 40°30'N, 72°40'W	Oct. 20–31, 1973	0.87 0.57 0.39 0.77 0.77 0.65
Cruise TR-152 31°20'N, 64°50'W 31°30'N, 65°20'W 32°00'N, 65°30'W 40°40'N, 66°30'W 41°30'N, 68°30'W 42°20'N, 66°40'W	May 9–19, 1974	0.16 < 0.1 0.39 < 0.06 < 0.04 0.05
Cruise TR-153 33°40'N, 57°00'W 34°30'N, 58°40'W 40°30'N, 70°00'W	May 25–31, 1974	0.11 0.58 0.04

*Midpoint of the collection track.

†The mean blank corresponded to 0.03 ng m⁻³ for a 1,000-m³ sample.

‡Range, mean and (number of samples).

toxaphene (Fig. 2c and d). Toxaphene was quantitated by comparing the sum of the peak heights in sample and standard chromatograms.

Atmospheric concentrations of toxaphene over the western North Atlantic are reported in Table 1. For several reasons we think that the majority of toxaphene in the air did not originate from Bermuda: (1) the Bermuda Department of Agriculture and Fisheries assured us that toxaphene has not been used on the island for the past 3–4 years; (2) the mean toxaphene concentration of Bermuda samples collected OSS (0.81 ± 0.45 ng m⁻³) (precision reported as s.d.m.) was little different from the mean of those taken when the wind blew ISS (0.72 ± 0.09 ng m⁻³); and (3), comparable levels were measured over the open

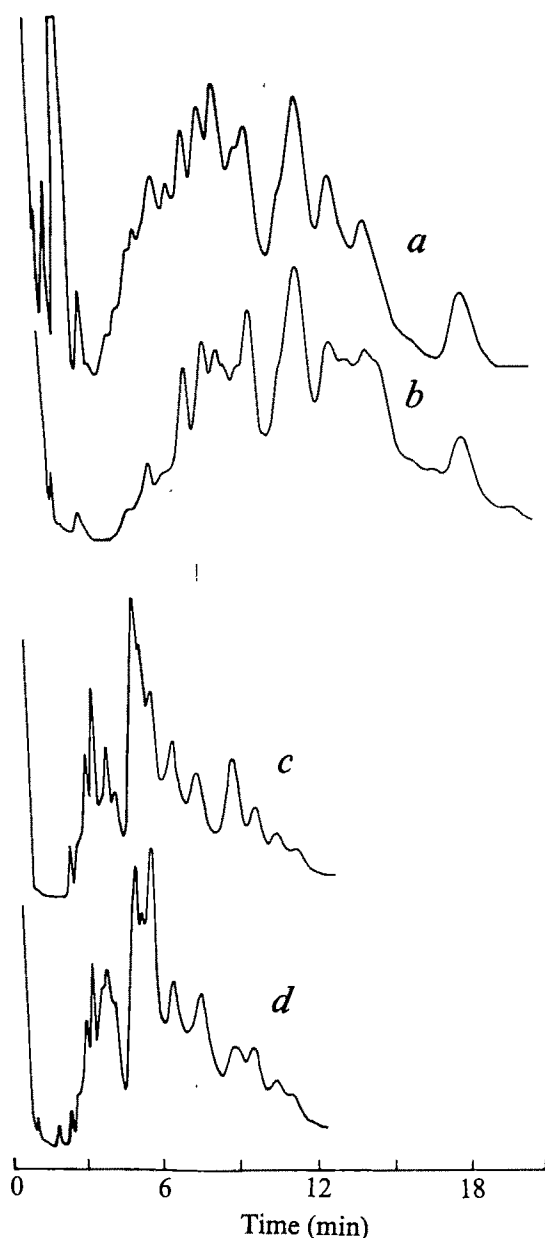


Fig. 2 Gas chromatographic identification of toxaphene in western North Atlantic air, 4% SE-30/6% QF-1 column. *a*, Bermuda air sample; *b*, toxaphene standard, 3 ng; *c*, Bermuda air sample, after dehydrochlorination; *d*, Toxaphene standard, 1.4 ng, after dehydrochlorination.

ocean, indicating that our Bermuda values were unlikely to be the result of local contamination.

Our mean levels of toxaphene in western North Atlantic air are about equal to or twice those of PCBs^{8,9}, and more than ten times higher than those of other pesticides so far reported in the marine atmosphere. These higher residues probably result from both the large scale use of toxaphene and its volatility. The mean toxaphene concentration (ng m^{-3}) in our 56 samples from Bermuda and RV Trident was 0.63, as against 0.024 for *p,p'*-DDT. We note that the ratio of these concentrations (26) is close to the ratio of the outdoor evaporation rates of the two pesticides (25) (ref. 3). A possible source of airborne toxaphene is the southern US cotton growing areas, where the bulk of this pesticide has been used in combination with DDT (up to 1973) or methyl parathion¹.

We thank the US Naval Underwater Sound Laboratory, Tudor Hill, Bermuda, for providing a site for the construc-

tion of the air sampling tower, and the University of Georgia Marine Institute, Sapelo Island, Georgia, for providing ship time on the RV Kit Jones. We are grateful to Paul DesLauriers and Ian Fletcher for their help in sample collection.

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New interglacial site at Sugworth

A PRELIMINARY account is given of a richly fossiliferous interglacial deposit of Middle Pleistocene age at Sugworth, near Oxford, UK.

Excavations for the A34 Abingdon bypass during 1972-73 revealed four fossil channels cut into the Kimmeridge Clay (Figs 1 and 2). The tops of the channels lie at 92 m OD, 40 m above the present level of the River Thames, and seem to represent a former meandering river trending roughly from north to south. In one section near Sugworth Lane (OS ref.: SP 513007; Fig. 2) the channel fill, approximately 180-200 m wide and up to 5 m deep, included organically-rich silts and sands passing laterally into sands and gravels and upwards into about 0.5 m of yellow silty clay. Across the channel fill an unconformable layer, 1-2 m thick, of pebbly sandy clay could be followed along the cutting for 100 m or so beyond the channel. This latter deposit has been mapped by the UK Geological Survey (Sheet 236) as 'Plateau Gravel' and 'Unbedded Drift', the first designation identifying it with the Plateau Drift of the Oxford region which most workers have regarded as wholly or in part of glacial origin¹.

Pebble counts and heavy mineral analyses of the channel sediments, the overlying pebbly clay, and several samples of Plateau Drift from the Oxford region, were compared in an attempt to establish their mutual relationships. Quartz, quartzite and sandstone, derived ultimately from the Bunter Pebble Beds, presumably of the Midlands, are major constituents of all three. Flint, derived from the Chalk, possibly when the scarp extended further north than it does today, is common throughout, but limestone of both Kimmeridge and Oolitic lithologies is abundant only in the channel sediments. In contrast, the channel sands and the overlying pebbly clay resemble each other and the Kimmeridge Clay

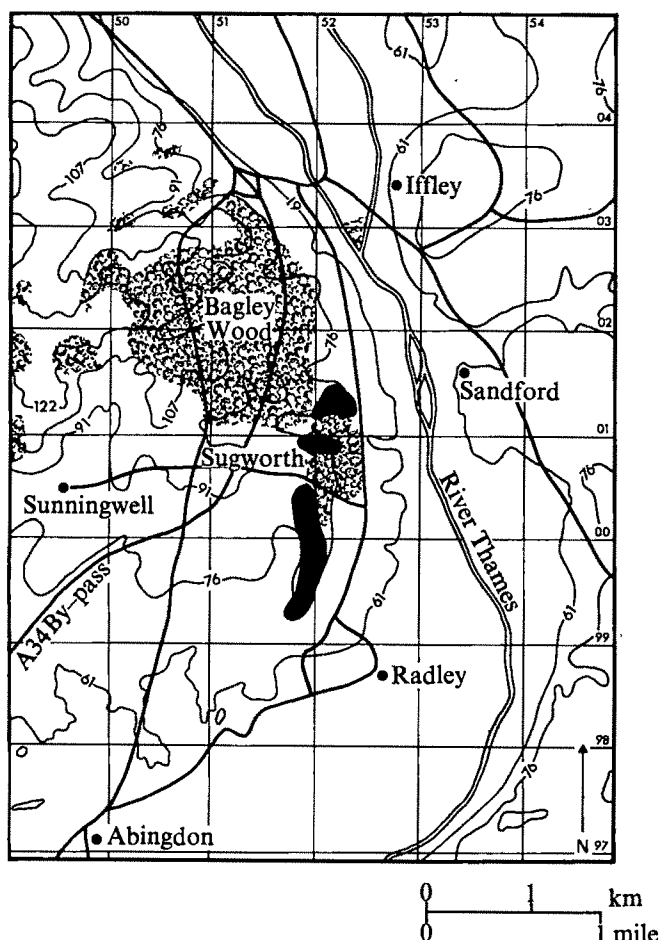


Fig. 1 Map showing location of Sugworth in relation to the Hanborough Terrace (black areas) and the modern River Thames.

in their zircon-dominated assemblage, whereas it has been shown that zircon is rare in the Plateau Drift².

The organic channel sediments are rich in molluscs, beetles and macroscopic plant remains, and vertebrates also occur. Unfortunately, well preserved pollen has not so far been recovered.

The macroscopic plant fossils represents aquatics (for example, *Callitriche* sp. and *Nuphar lutea*), plants characteristic of wet soil (for example, *Lycopus europaeus*, *Eupatorium cannabinum* and *Filipendula ulmaria*), Cyperaceae (sedges), open ground herbs (*Atriplex* sp., *Chenopodium* sp., *Rumex* sp., *Plantago major* and *Leontodon autumnalis*), forest trees (*Acer campestre*, *Alnus glutinosa*, *Carpinus betulus*, *Abies* sp. and *Picea* sp.) and shade tolerant herbs (for example, *Moehringia trinerva*). The logs collected from the deposit are mostly *Ulmus*, together with *Quercus* and a Rosaceae taxon, probably *Crataegus* (hawthorn).

The molluscs from the basal 3 m include many moving water/river species (for example, *Unio crassus*, *Ancylus fluviatilis*, *Valvata piscinalis*, *Bithynia* sp., *Pisidium amnicum* and *P. henslowanum*) reflecting conditions of deposition in a large river. Shells washed in from other habitats include species of reed swamp and fen (for example, *Succinea* spp., *Carychium minimum*, *Cochlicopa lubrica*, *Vertigo angustior* and *Hygromia hispida*) together with a 'slum' group indicative of the presence of small bodies of water, liable to dry up (*Sphaerium lacustre*, *Planorbis leucostoma* and *Pisidium obtusale*), snails of drier rather open terrestrial habitats (for example, *Vallonia costata*) and those favouring a denser forest cover (for example, *Azeca* cf. *menkeana* and *Discus rotundatus*).

Vertebrates include fishes (for example, *Esox lucius*), amphibians (*Rana* sp. and/or *Bufo* sp.) and small mammals (*Sorex* cf. *savini*, *Talpa* sp., *Clethrionomys glareolus*, *Mimomys savini*, *Microtus* sp. and *Apodemus* (sylvaticus group)). Remains of large mammals are few but include a tooth of the rhinoceros *Dicerorhinus etruscus*. The predominance of remains of woodland rodents *Clethrionomys* and *Apodemus* over those of the grassland vole *Microtus* contrasts with assemblages from West Runton³, where the reverse is true, and suggests that forest grew close to the river.

The extensive beetle fauna includes a number of species such as *Hypulus quercinus*, *Eremotes lignarius* and *Dryophthorus corticalis*, which live in deciduous woodland. The general picture, therefore, is of a large river flowing through fairly dense, mixed oak forest, probably with locally developed, herb dominated communities and marshy areas on the floodplain. The channel sediments seem to have accumulated on the inside of a meander.

The faunal and floral evidence strongly indicates a temperate climate and there is no doubt that the deposit is of interglacial age. Some of the plants (for example, *Najas minor*, *Abies* and *Trapa natans*) occur today in central or

Table 1 Known stratigraphical ranges (England) of certain taxa recorded from Sugworth

	Pre-Cromerian	Cromerian*	Hoxnian	Ipswichian
Plants				
<i>Abies</i> sp. ⁸		+		+
<i>Azolla filiculoides</i> ⁹⁻¹⁰	+	+	+	
<i>Trapa natans</i> ⁸		+		+
Molluscs				
<i>Nematurella runtoniana</i> ¹¹		+		
<i>Valvata goldfussiana</i> †		+		
Mammals				
<i>Mimomys savini</i> ¹²	+	+		
<i>Dicerorhinus etruscus</i> ¹²	+	+		

* Cromerian records are from the type site at West Runton, Norfolk⁷.

† B. W. Sparks, personal communication.

southern Europe. The beetle fauna includes a substantial proportion of species no longer living in Britain but all found further south in Europe today. Examples of this group are *Oxytelus opacus*, *Pelochares versicolor* and *Valgus hemipterus*. There is thus no doubt that the interglacial experienced warmer summer temperatures than the south Midlands of the UK today.

The insect fauna cannot be compared with a definitive Cromerian assemblage as nothing of that age has yet been fully investigated. It is significant, however, that it bears no resemblance to Hoxnian faunas of the Midlands which have been closely studied (ref. 4, and Kenward, unpublished), but there is a marked resemblance to the Ipswichian fauna from Trafalgar Square, London. Nonetheless, a correlation of the Sugworth deposits with the Ipswichian is surely precluded on stratigraphical grounds and on consideration of other fauna and flora with a restricted distribution in time. The known stratigraphical ranges of certain of the Sugworth taxa in deposits of the last three British interglacials (Table 1) cumulatively point to a Cromerian age for the Sugworth channel deposits. More specifically, the abundance of *Carpinus* fruits and the remains of *Abies* and *Picea* suggests that the deposit belongs to zone CrIII. A radiocarbon assay gave an 'infinite' date (Birm 381).

The Sugworth deposit lies topographically well above the Hanborough Terrace (see Fig. 1) which has been ascribed both to the Hoxnian Interglacial and, more recently, to an early part of the Wolstonian⁵. It must, therefore, be at least as old as the Hoxnian and is more

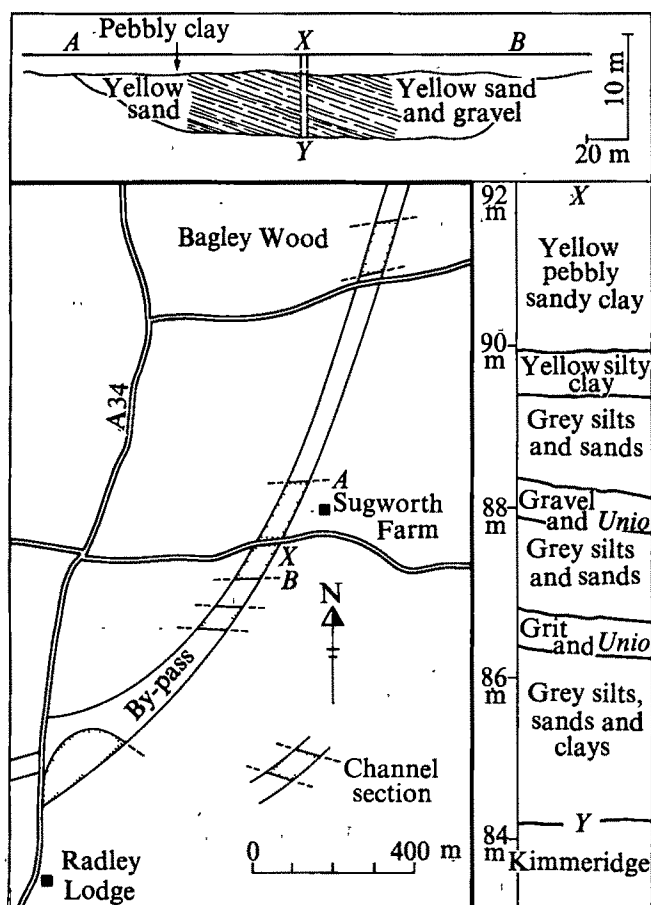


Fig. 2 Location of the channels: idealised section (A-B) of the channel (vertical exaggeration, $\times 5$) with fossiliferous deposits obliquely shaded; detailed stratigraphy, X-Y.

probably even older than that. The evidence that it is Cromerian has wide implications for the Pleistocene stratigraphy of the Oxford region⁸. Of the coarse constituents of the channel deposits, only the Bunter pebbles pose a serious problem since it is possible to suggest a local or near-local source for the other types. If the Bunter pebbles are derived from the Plateau Drift, then that deposit is pre-Cromerian and the pebbly clay which overlies the channel is not Plateau Drift, though it may be a solifluction deposit from it. Since no glacial deposits earlier than the Anglian stage (that is, post-Cromerian) have so far been reliably identified in Britain⁷, the whole question of the mode of deposition of the Plateau Drift, glacial or otherwise, would need clarification were a pre-Cromerian age accepted. If, on the other hand, the pebbly clay above the channel is considered to be Plateau Drift, then that deposit remains in the Anglian stage. In that case, however, a new problem arises: by what mechanism did the Bunter pebbles arrive at Sugworth if earlier ice-transport is ruled out? Possibly, since the channels seem to be part of a river system flowing roughly from the north (assuming that they are indeed relics of a very old river system greatly at variance with the modern pattern) the Bunter pebbles could have been transported from the NNW by fluvial processes. In any case, a minimum distance of transport of 100 km is demanded.

In a wider context Sugworth is important as the only known British open site of Cromerian age outside East Anglia.

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Adverse effects of maternal alcohol consumption on pregnancy and foetal growth in rats

JONES *et al.* have recorded several combined aspects of dysmorphogenesis, severe physical growth retardation and mental deficiency in the human and named it "foetal alcoholic syndrome", suggesting the defects to be associated with offspring of chronically alcoholic mothers¹. Sandor and Amels² found that ethanol seemed to produce distinct teratological effects in Wistar rats and chick embryos when it was administered intravenously to the pregnant mother or the hen at critical periods in gestation.

In spite of the increasing influence of alcoholism in Western society and the trend toward increased concern for human prenatal health care, no systemic model—human or animal—has been designed to ascertain the adverse effects of maternal alcoholism on the outcome of pregnancy and the growth of the developing foetus. Adequate data in this field are lacking³. This study proposes a simple experimental model to elucidate some information in this area.

Thirty-seven inbred Sprague-Dawley female rats (each about 200 g; Bio-Breeding Laboratories, Ottawa, Canada) were randomly divided into two treatment and one control group, and housed individually in wire cages. For a 5-week period before mating, 13 females (group A) received ethanol in 30 g per 100 ml water as their only available fluid, and a balanced powdered diet (Table 1) *ad libitum*. After 1 month, blood samples were taken from the tails of 10 group A animals for the determination of blood alcohol levels according to the alcohol dehydrogenase method. The

Table 1 Composition of powdered diet (4.2 calorie g⁻¹) fed to all three groups of studied animals

Component	% (by weight)
Casein	20
Corn oil	10
Cornstarch	64
Alphacel	1
Mineral salts	3
Vitamin preparation	2

pair-fed group (B) received water *ad libitum* and were fed a diet isocaloric with that of the group A animals. The isocaloric diet was determined on the basis of the average daily caloric intake of group A animals, in the consumption of both the powdered diet and ethanol. The control group (C) received food and water *ad libitum*.

Food and fluid intake records were kept for all three groups. At mating, three females with one male of the same age, source and strain were placed in each standard plastic breeding cage overnight. All these groups of animals were allowed water during the nights on which they were mated. The presence of sperm cells in vaginal smears taken the following morning was taken as positive indication of pregnancy. Group A animals continued to receive alcohol and the intake of group B animals was regulated accordingly throughout the gestation period. All offspring were examined, weighed and counted on the day of birth.

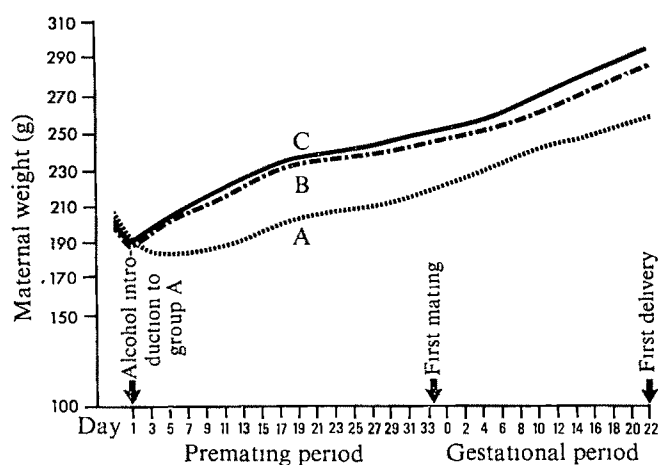
Table 2 Copulation and delivery rates of alcohol-fed, pair-fed and control group females

Group	Number	Positive smears	Successful deliveries
A	12	10 (83%)	5 (50%)
B	13	11 (85%)	10 (91%)
C	11	8 (75%)	7 (88%)

A, Alcohol-treated; B, pair-fed to alcohol-treated; C, control.

Throughout the study period, the daily weight gain of group B females paralleled closely that of control females. Group A animals, after an initial sharp decline in weight with change in environment and start of ethanol administration, gained weight in a pattern similar to that of control and pair-fed females but the group mean weight remained at least 20 g below those of groups B and C (Fig. 1). The mean serum alcohol level of ten group A animals was 61 ± 23 mg %.

No significant difference was found among the three groups with respect to numbers showing positive indications

Fig. 1 Maternal daily weight changes during prenatal and gestational periods in three groups of studied animals. A, Alcohol-fed; B, pair-fed to group A; C, control.

of pregnancy; but whereas 88 and 91% of control and pair-fed females, respectively, which copulated, produced litters, only 50% of group A mothers known to have copulated, delivered litters (Table 2). The average litter size of alcohol-treated mothers (7.2) was significantly lower than the mean litter size of either pair-fed or control mothers in this study. The mean birth weight (4.58 g) of group A litters was also lower than that of litters of group B or C animals (Table 3, Fig. 1). Gestation period did not vary among the three groups; all litters delivered at 21 to 22 d *post coitum*.

In addition to small size, offspring of group A mothers exhibited microcephaly, cracked, dry, loose skin, reddened areas on the head and body, and a generally shrivelled appearance.

Table 3 Birth weights of progeny and litter size of alcohol-fed, pair-fed and control females

Group	Mean birth weight (g)	No. of animals per litter at birth
A	4.58 ± 0.23* (N = 34)	7.2 ± 3.4 (n = 4)
B	6.00 ± 0.61 (N = 87)	10.5 ± 3.1 (n = 10)
C	5.88 ± 0.64 (N = 80)	11.7 ± 2.5 (n = 7)

* Mean ± s.d.

N, Total number of offspring; n, total number of litters; A, alcohol-treated; B, pair-fed to alcohol-treated; C, control.

Toews and Lee studied the effect of maternal dietary deprivation of rats on growth and development of progeny⁴, and found that there was no significant effect of nutritional deprivation on litter size, although the mean weight of the progeny was significantly decreased (5.89 g for the offspring of nutritionally restricted animals compared with 6.60 g for offspring of control animals). The difference between the means was 0.71 g. In the present study, the mean weight of progeny in control litters was 5.88 g, whereas that of alcohol-fed litters was 4.58 g. This striking difference of 1.30 g indicates the greater effect of maternal alcohol consumption, rather than dietary restriction, in controlling the size of progeny. Although mothers which were pair-fed to alcohol-ingesting mothers received a somewhat restricted intake of 60 calorie d⁻¹, when compared with *ad libitum* intake of control mothers at 68 calorie d⁻¹, the data show no significant difference between control and pair-fed groups with respect to any of the parameters studied.

Our results indicate that maternal consumption of alcohol before and during pregnancy markedly decreased the size and number of progeny produced. These effects do not seem to be calorie-dependent but rather to be direct effects of the ingestion of alcohol. The simple experimental model used here can readily be applied to other investigations of the adverse effects of maternal alcohol consumption on morphogenesis, foetal growth, postnatal growth and behavioural development of the offspring.

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Alcohol prolongs time course of glare recovery

SUDDEN changes in environmental light levels require the eye to readjust to achieve the same sensitivity to target contrast at the new level, a process which takes many seconds or minutes when the new environment is considerably dimmer than the previous level. During this recovery time, the eye is relatively blind to fine detail. We show here that relatively low doses of alcohol significantly prolong recovery times following bright light exposure; these changes can be seen for several hours following alcohol ingestion.

When the integrity of the retina is disturbed by drugs or disease, the adaptation process is retarded and the recovery of contrast sensitivity is prolonged^{1,2}. Halothane anaesthesia has been shown to markedly prolong the retinal dark adaptation process³. Alcohol, a commonly used drug, is also known to have a direct effect on the human retina; the b wave^{4,5} and the c wave⁶ of the dark-adapted electroretinogram are altered by alcohol at levels commonly encountered in social drinking.

Nine young male subjects (aged from 20 to 28) participated in a replicated 3 × 3 crossover experiment which was run double blind. Two alcohol doses (0.5 and 1.0 ml per kg body weight) and a placebo were used. Alcohol in the form of 95% ethanol was diluted with fruit juice so that the total volume (ml) was three times the subject's body weight (kg). The drink was consumed through a straw from a lidded paper cup which also contained two ice cubes. To minimise olfactory cues to the presence of alcohol, 2 drops each of ethanol and eucalyptus oil were placed on the lid of the cup. Twenty minutes were allowed for the consumption of the drink. Blood alcohol level (BAL) was estimated before drinking and at intervals after drinking using the Intoxilyser (Omicron Systems, Palo Alto) which

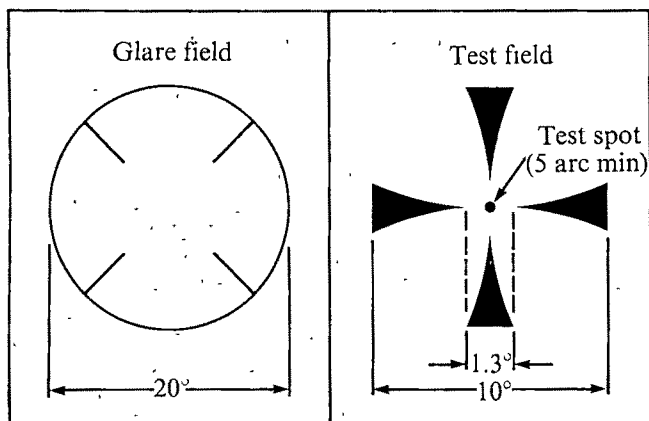


Fig. 1 Glare and test field configuration: Subjects preadapted for at least 5 min to the low photopic luminance levels of the laboratory. Aided by four thin diagonally oriented reference lines, the subject fixated the centre of a 20° circular adapting field of 5.6×10^4 cd m⁻² with the right eye. Immediately following a 10-s exposure to the high intensity adapting field, the subject fixated the centre of the stimulus configuration where a 5 arc min test spot was intermittently presented (125-ms flashes at 4 Hz) on a 22.6 cd m⁻² background. The contrast of the 5 arc min test spot was controlled by the subject. When he had recovered contrast sensitivity and detected the target, he operated a switch to reduce the target contrast a further fixed step below his threshold. The contrast levels of the test spot, 5.34, 2.00, 1.45, 0.99 and 0.64, were chosen to give approximately equal time periods between each recovery point. Four sets of measurements were taken with 5 min allowed between sets for complete recovery from the adapting luminance level. Photographs of the left eye were taken on five subjects 5 s before and 5 s into the exposure of the high intensity adapting stimulus for the first trial of each test period. Measurements of pupil size were made from the projected negatives.

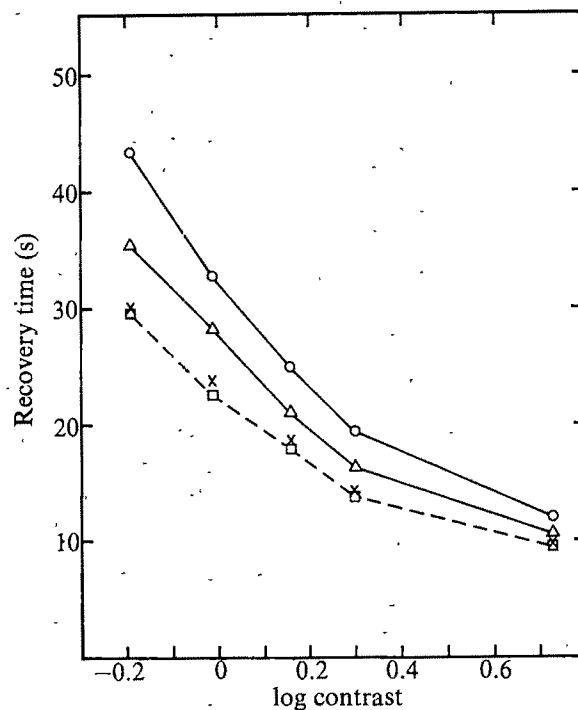


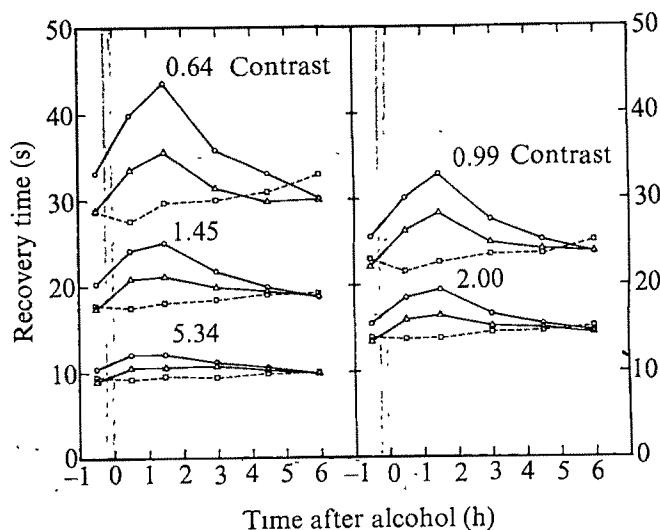
Fig. 2 The time course of adaptation for placebo and alcohol 90 min after drinking. 1.0 ml per kg body weight of 95% ethanol (○); 0.5 ml per kg body weight of 95% ethanol (△); placebo (□). The dose relationship for the alcohol doses is evident in the displacement of the two alcohol treatment curves. The insensitivity of the measure to placebo treatment is also shown here: the mean pre-drink recovery times collapsed across test days (×) fall on the curve generated by the placebo recovery times.

estimates BAL using infrared spectroscopy of a breath sample.

Details of the stimulus presentation are shown in Fig. 1. Subjects practised the task to achieve stable performance before participation in the experimental sessions. On a given experimental day, they were tested before drinking and 30, 90, 180, 270, and 360 min after drinking. At each of these test times, blood alcohol levels and subjective 'high' ratings were ascertained by a second experimenter. Subjects rated their 'high' on a scale of zero to 100, where zero was sober and 100 was as high as a subject had ever been on alcohol.

The time course of adaptation following a 10-s exposure to a uniform bright field is significantly retarded after alcohol

Fig. 3 Group mean recovery time (s) as a function of time after drinking. These functions describe the time course of the alcohol induced light adaptation changes for the five contrast levels and each drug condition. The shaded area indicates the time of alcohol ingestion. Symbols as in Fig. 2.



ingestion. Figure 2 shows the group recovery time to test spots of different contrast for placebo, 0.5, and 1.0 ml of 95% ethanol per kg body weight doses 90 min after drinking. The group recovery times for the low alcohol (0.5 ml kg⁻¹) and high alcohol (1.0 ml kg⁻¹) doses are all increased significantly ($P < 0.02$ Walsh test) when compared with the corresponding pre-drink values. For the 1.0 ml kg⁻¹ alcohol dose, group mean recovery times are delayed by between 30 and 50% compared with the corresponding placebo values.

The alcohol induced increase in recovery times is apparent in the first measurement period (30 min), peaks at the 1–2 h point, and thereafter declines to reach pre-drink values approximately 6 h after drinking (Fig. 3). There is a significant systematic increase ($P < 0.01$, Friedman two-way analysis of variance) in glare recovery time at all five contrast levels for the high dose of alcohol (1.0 ml kg⁻¹); for the low dose of alcohol (0.5 ml kg⁻¹) the shift is statistically significant ($P < 0.05$) for two contrast conditions (2.00, 0.99). Recovery times at all contrasts remain elevated 3 h after drinking.

The alcohol induced increases in recovery times are dose related. The dose relationship is clearly evident 90 min after drinking (Fig. 2). Furthermore, it is evident from Fig. 3 that a dose relationship exists for at least 3 h after drinking when the mean BALs are quite low (0.01 and 0.05 g% for the 0.5 and 1.0 ml kg⁻¹ doses, respectively).

Relatively low doses of alcohol produce significant dose-related delays in the recovery of contrast sensitivity following exposure to intense light levels. The relationship between BALs and the increase in recovery times is illustrated in Fig. 4. Both the mechanisms of the alcohol induced change in the adaptation process and the consequences for a wide range of practical tasks deserve attention.

How does alcohol ingestion produce the increased recovery times? A number of preretinal factors could be involved, including pupil size changes, pupil response to the adapting stimulus, accommodative error, and accuracy of eye fixation.

Fig. 4 Mean recovery time to targets of five contrasts as a function of blood alcohol level. The range of blood alcohol levels was divided into five equal intervals and the corresponding glare recovery times were averaged within these intervals. The number of measures contributing to each point is indicated along the abscissa. Contrast levels: 5.34 (□), 2.00 (△), 1.45 (○), 0.99 (◆), and 0.64 (▽).

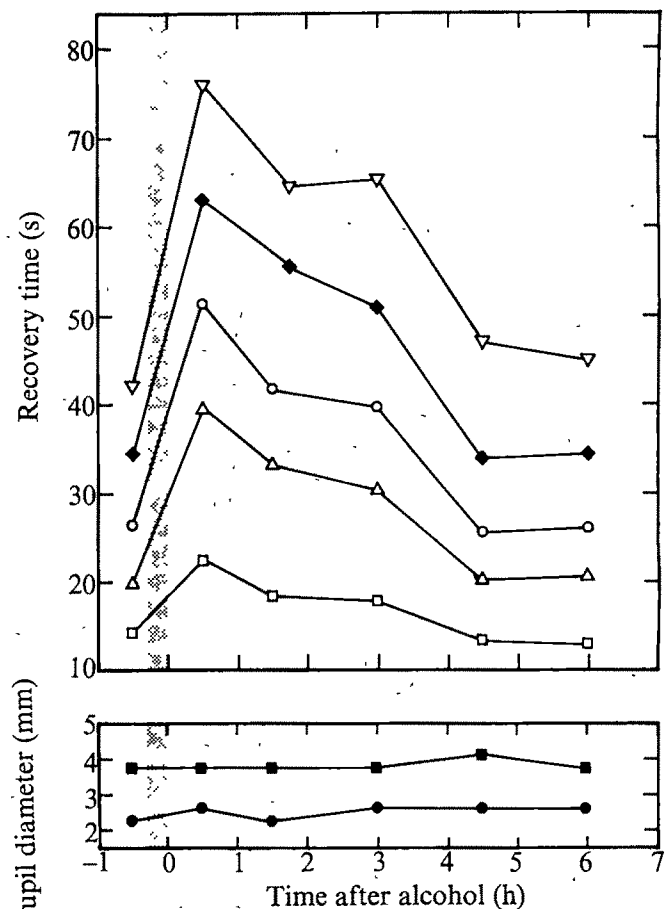
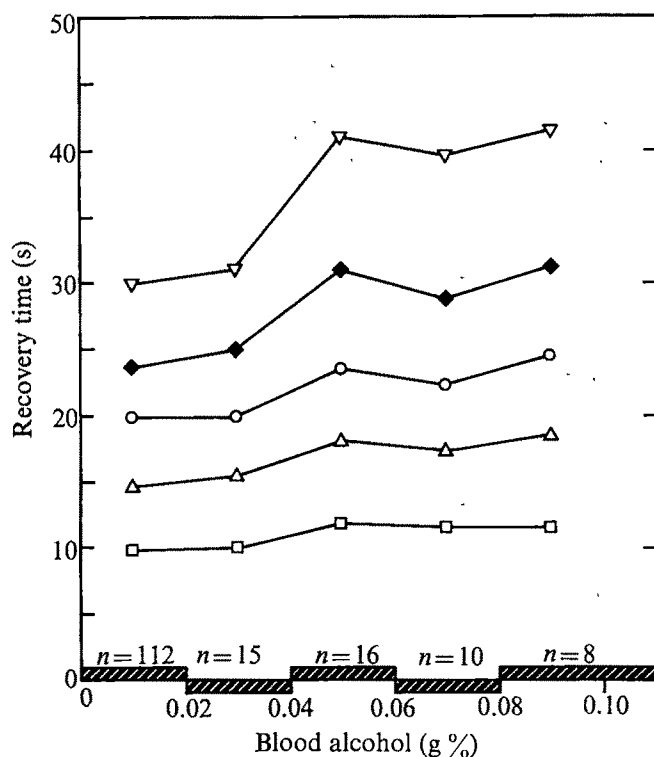


Fig. 5 Recovery time (s) to five different contrasts as a function of time after alcohol (1.0 ml kg⁻¹); subject J.W. Contrast levels: 5.34 (□), 2.00 (△), 1.45 (○), 0.99 (◆), and 0.64 (▽). Shown below is pupil diameter (mm) for this subject before exposure to the glare field (■) and during exposure to the glare field (●) as a function of time after alcohol.

Alcohol induced changes in pupil size could be expected to influence the recovery time to fixed contrast targets. Pupil size showed no consistent variations, however, either with time after drinking or across drug treatments. Figure 5 shows the results for a subject whose glare recovery times were markedly altered by alcohol at all contrast levels. The pupil sizes before and during glare exposure, 90 min after drinking, are no different from their pre-drink counterparts; at this same time there is an average of 54% increase in glare recovery time for the subject. Inaccuracies of accommodation to the test target or inexact foveal fixation could produce longer recovery times. It is unlikely, however, that either of these factors was significant in these experiments. Perhaps the most important evidence for this statement is that visual acuity was unaltered by identical alcohol doses in another experiment on 10 young adult males (Adams *et al.*, unpublished).

Alcohol has a direct effect on the human retina⁴⁻⁶. Further, Raskin *et al.* have shown that alcohol slows dark adaptation and the resynthesis of photopigment in albino rats; their evidence suggests that this is due to the inhibition of a catalyst thought to be required for the oxidative resynthesis of retinaldehyde from retinol⁷. Although some of the evidence is indirect, we feel that preretinal factors and central mechanisms, which have little or no role in light adaptation⁸, are not significantly involved in the alcohol induced slowing of adaptation in our experiments. The results of Raskin *et al.* raise the interesting possibility that alcohol may be inhibiting the resynthesis of photopigment, an hypothesis that needs to be tested by measuring cone pigment kinetics.

The increased recovery time produced by alcohol intoxication must be viewed as critical from a practical point of view,

regardless of the mechanism. The period of recovery is a period of relative blindness for the individual and, as such, is potentially hazardous. Soon after sunrise and just before sunset the sky may act as an extended glare source for the automobile driver. The luminance levels in these conditions may be as high as those experienced by the subjects in our experiment⁹. In certain circumstances a driver will intermittently view the bright sky or be subjected briefly to high luminance glare from light scattered by the windshield. Following glare, important features of the driving environment must be seen against average background levels similar to those which we used. The possible consequences of an additional 30–50% delay in seeing critical detail in such circumstances are obvious.

We have demonstrated alcohol induced delays in glare recovery which we believe to be occurring at the retinal level. These delays have been demonstrated at surprisingly low BALs (approximately one cocktail on an empty stomach) and are dose related. The luminance parameters of the test are similar to luminance levels sometimes encountered in practice.

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Macrophage proliferation induced *in vitro* by a lymphocyte factor

MACROPHAGE proliferation occurs *in vivo* as a component of the inflammatory response¹ and particularly intense proliferation is characteristic of the delayed hypersensitivity reaction to infection with facultative intracellular pathogens^{2–4}. The thymus-derived (T) lymphocyte is considered responsible for the expansion of the reticuloendothelial system in this type of infection⁵. Although the mechanisms by which lymphocytes induce macrophage proliferation and activation remain unclear, a number of lymphocyte-produced soluble mediators which influence the functions of macrophages have been described, including a biochemically distinct migration inhibitory factor (MIF) and chemotactic factor (MCF)⁶. The speculation that a lymphocyte-produced mitogenic factor may be responsible for the induction and maintenance of proliferation of macrophages prompted the present experiments.

There is evidence that macrophages proliferate *in vitro* in conditioned media or in the presence of a fibroblast-derived growth factor⁷ and two recent reports^{8,9} indicate that inflammatory exudates contain a factor which induces *in vitro* macrophage replication. Here we confirm our preliminary work¹⁰ in demonstrating that guinea pig lymph node lymphocytes in conditions shown to produce intense delayed hypersensitivity produce a soluble factor which induces guinea pig macrophages to proliferate *in vitro*.

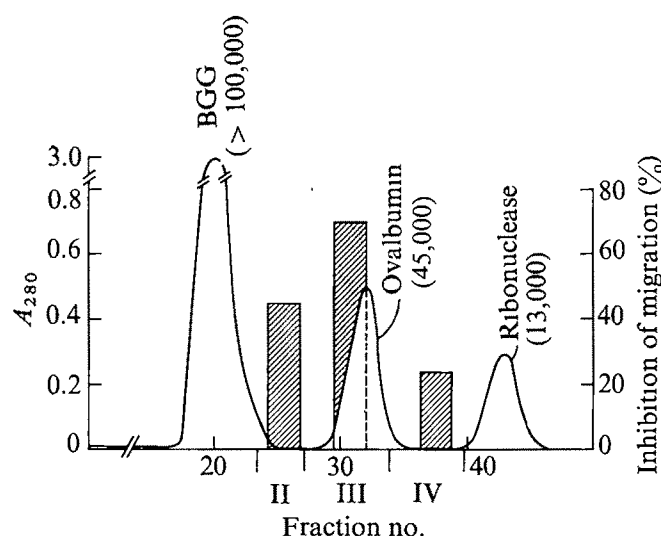


Fig. 1 Sephadex column chromatography of lymphocyte supernatants. P and R supernatants were dialysed, lyophilised and subjected to Sephadex G-100 (superfine) column chromatography as described¹². Fractions were pooled and assayed for MIF activity on peritoneal macrophages at concentrations equivalent to the undiluted supernatants. MIF activity is expressed as percentage migration inhibition ([P/R] × 100) and depicted as columns. Markers for molecular weight estimation are depicted as curves. Molecular weights are given in parentheses.

Soluble mediators were prepared as previously described by us for the generation of MIF-rich supernatants¹¹. Briefly, Hartley guinea pigs were immunised with bovine gamma globulin (BGG) in Freund's complete adjuvant. Between days 14 and 17 regional lymph nodes were harvested and the cells collected and incubated for 24 h at a concentration of 15×10^6 per ml Eagle's MEM without serum, with and without BGG (1 mg ml^{-1}). After incubation, supernatants were collected and antigen was added to control supernatants. In these experiments, supernatants preincubated with antigen (P) were compared with controls reconstituted with antigen (R). Alveolar and oil-induced peritoneal macrophages from non-immunised animals were used in the capillary tube migration inhibition assay as described¹¹. Supernatants having potent MIF activity at a 1:2 dilution on the peritoneal macrophage (migration inhibition of 60–80%, that is, [P migration/R migration] × 100) were used in these experiments.

Peritoneal exudate cells and alveolar macrophages were incubated in glass tubes, Lab-Tech chambers or Falcon microculture plates for 30 min at 37°C to allow for the formation of monolayers. Non-adherent cells were removed by decanting followed by three washes in Hanks' balanced salt solution. Monolayers (>98% macrophages) were incubated for varying times in medium containing 20% foetal calf serum, 1% *L*-glutamine, and antibiotics. The incorporation of $0.5 \mu\text{Ci}$ of ^3H -thymidine (20 Ci mmol^{-1}) during

Table 1 DNA synthesis in macrophage monolayers

	Lymphocyte-produced mediator-rich supernatant dilutions	
	1:4	1:2
Alveolar macrophage	3.0 ± 0.3 (9)	4.2 ± 0.4 (12)
Peritoneal macrophage	2.8 ± 0.2 (33)	4.9 ± 0.4 (12)

Because of considerable variation in the background thymidine incorporation between alveolar and peritoneal macrophages and between one animal and another, the thymidine incorporation data for macrophage monolayers incubated for 5 d are expressed as the ratios (P supernatant/R supernatant) \pm s.e.m. of the ratios (number of samples). Mean c.p.m. thymidine incorporation for R supernatants were 7,300 c.p.m. for alveolar macrophages and 3,000 for peritoneal macrophages.

a terminal 24-h pulse was assayed. The tube cultures were processed as described¹². The microculture plates were twice freeze-thawed at -70°C to ensure release of the cells from the monolayer then processed by a multiple automatic sample harvester (MASH, Hiller), followed by liquid scintillation spectrometry.

Monolayers of macrophages incubated in MIF rich supernatants showed significant increases of thymidine incorporation peaking on the average at 5 d of culture. Table 1 shows the results of five experiments in which peritoneal and alveolar macrophages were incubated for 5 d in the presence of 1:2 and 1:4 dilution of P and R supernatants. Negligible decreases in thymidine incorporation were observed with R supernatants compared with media controls. The P supernatants consistently increased thymidine incorporation in both alveolar and peritoneal macrophages. The ratios of stimulation are significant ($P < 0.001$).

Table 2 DNA synthesis and content of macrophage cultures

	48 h		120 h	
	Thymidine incorporation	DNA content	Thymidine incorporation	DNA content
P fraction III	2,839	17.0	20,207	24.8
R fraction III	3,124	17.0	1,381	14.4
Medium control	8,430	18.2	668	16.8

Thymidine incorporation was assayed in microculture in which 1×10^5 peritoneal macrophages were allowed to attach for 30 min at 37°C . Monolayers were washed thrice and incubated for 48 or 120 h. Cultures were terminated with a 24 h ^3H -thymidine pulse (0.5 μCi) and assayed for thymidine incorporation by MASH and liquid scintillation spectrometry. DNA content was assayed in parallel using tube culture in which 5×10^5 macrophages were used for monolayer formation. DNA content of the cultures was measured by the method of Burton¹⁴. Data are expressed as mean c.p.m. or μg of DNA for triplicate determinations in a single experiment.

The crude supernatants contained antigen and possibly small amounts of antigen-antibody complex¹⁵, and since antigen-antibody complexes have been shown to induce macrophage proliferation¹⁶, we fractionated the supernatants on Sephadex G-100 and found that the major portion of the migration inhibitory activity eluted from the column in fractions of estimated molecular weight range of 35–60,000 (fraction III). Similarly, most of the mitogenic activity eluted in this fraction (Fig. 1). Figure 2 shows a time course of the effects of crude supernatants (1:2 dilution) and Sephadex fractions III run at a concentration corresponding to 1:2 and 1:4 dilution of the original supernatants. The increased mitogenic ratio of the supernatants observed in Fig. 2 compared with those given in Table 1 resulted from improving of the culture conditions, particularly a decrease of concentration of CO_2 from 5% to 1%. We have also observed mitogenic activity in fractions eluting before ovalbumin included in fraction II. These results suggest that the mitogenic factor and MIF may represent the same factor; however, we have found that by performing extensive vacuum dialysis of the supernatants before column chromatography (30 h resulting in a 40-fold concentration), we could deplete MIF activity to $< 20\%$ migration inhibition without significantly diminishing the activity of the mitogenic factor. This observation indicates that the mitogenic factor is distinct from MIF.

Table 2 compares the effects of Sephadex P and R fractions III run at a concentration equivalent to an undiluted supernatant and a medium control on thymidine incorporation and DNA content at 2 and 5 d of culture and demonstrates that the mitogenic factor (P fraction III) increases both the thymidine incorporation and DNA content between 48 and 72 h of culture in contrast to the R and medium controls. Microscopic analysis of monolayer cultures during the period of incubation indicated that, compared with R Sephadex fractions, monolayers exposed

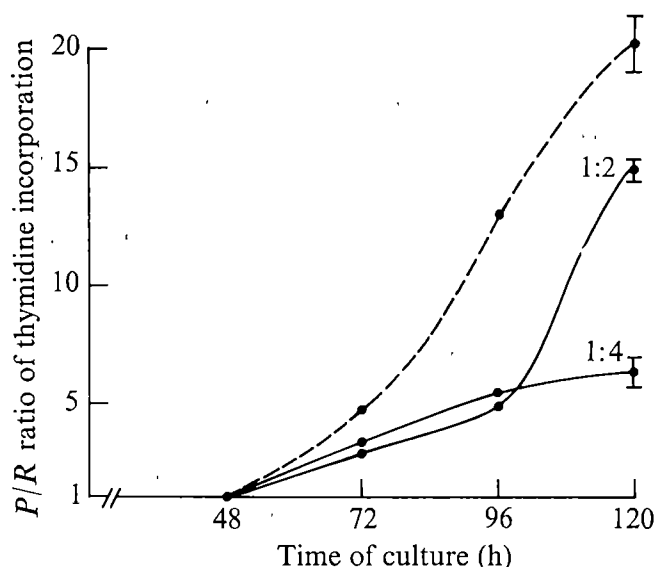


Fig. 2 Time course of mitogenic factor action on macrophage monolayers. P and R supernatants diluted 1:2 were assayed for mitogenic activity on peritoneal macrophage monolayers over 5 d of culture (-----). Comparable supernatants were fractionated on Sephadex G-100 and pooled fractions III (see Fig. 1) were assayed for mitogenic activity at concentrations corresponding to dilutions of the original supernatants of 1:4 and 1:2 (———). Mitogenic activity was determined by thymidine incorporation. The data are expressed as the ratio of P/R thymidine incorporation.

to P fractions containing the mitogenic factor without MIF activity showed no gross differences in the number of adherent cells at plating and during the first 24 h of incubation and marked increases in the number of cells in association with the peak of thymidine incorporation. In an experiment in which the peak thymidine incorporation occurred at 96 h, mean cell counts for monolayers incubated with P fraction III and R fraction III were 47 and 18 cells per high powered field respectively and autoradiographic analyses of cells incorporating ^3H -thymidine were 22.1% and 9.8% labelled cells respectively. The increase in the number of labelled cells having the morphology of macrophages and containing vacuoles filled with oil used to induce the exudate indicate that macrophages were the cells proliferating.

These experiments indicate that lymphocytes in immunological conditions designed to produce intense delayed hypersensitivity produce on re-exposure to antigen *in vitro* a soluble factor which induces the proliferation of macrophages in monolayer cultures. Column chromatographic purification of this factor indicates that this factor elutes with an estimated molecular weight comparable with migration inhibitory factor. Vacuum dialysis before column chromatography indicates that the two factors are different. The factor is also distinct from antigen-antibody complexes, a recognised mitogenic influence on macrophages. On the basis of molecular weight estimation, the factor would seem to be distinct from a lymphocyte produced factor called blastogenic factor (molecular weight 20,000) which is mitogenic for lymphocytes^{15,16} and from a fibroblast-produced factor (molecular weight $> 100,000$) which is mitogenic for peritoneal macrophages⁷. The relationship of this factor to that which induces proliferation in uncharacterised peripheral blood cells remains to be determined. On the basis of comparisons of estimated molecular weight^{17,18}, we estimate that the factor is similar to lymphocyte-produced colony stimulating factor (CSF) which acts on macrophage precursors; CSF has, however, been shown to stimulate colony formation in peritoneal exudate cells only after a delay of 10–14 d and then only in a small fraction of the cells (5%)^{19,20}. To our knowledge, this repre-

sents the first demonstration of lymphocyte-produced macrophage mitogenic factor (MMF) acting on mature macrophages.

The experimental protocol used to elicit MMF offers strong presumptive evidence for its production by T lymphocytes; but whether other cells, for example B lymphocytes, also produce it remains to be determined. Its selectivity of action on target cells remains to be established as well. The biological relevance of MMF seems to be as a participant in the expansion of the expression of the efferent limb of cellular immune response and perhaps in the process of macrophage activation.

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DNA present in multilayer rosette formed by lymphocytes stimulated with phytohaemagglutinin

ROGERS *et al.*¹ have reported that human lymphocytes of peripheral blood cultured in the presence of phytohaemagglutinin (PHA) undergo blast formation and synthesise DNA. This newly synthesised DNA which is excreted into the medium is reported to have a high molecular weight. The release of DNA by the cells is selective, as experiments with ¹⁴C-uridine indicated that RNA was not lost into the culture medium. The excretion of DNA by lymphocytes has also been noted by Olsen and Harris². Sarma and Rutman³ also found that PHA-stimulated lymphocytes incorporated labelled thymidine into small fragments that were then converted to high molecular weight material. Subsequently, the labelled thymidine reappeared in light fragments that were then released into the medium in an acid-precipitable form. It has also been reported that the human lymphocytes of peripheral blood are able to bind to sheep red blood cells (SRBC) in a rosette-forming cell⁴ when incubated together in stringent temperature conditions. In view of these findings the release of DNA synthesised by lymphocytes has been studied in connection with rosette formation.

Autoradiographs from the dry (labelled lymphocytes) and

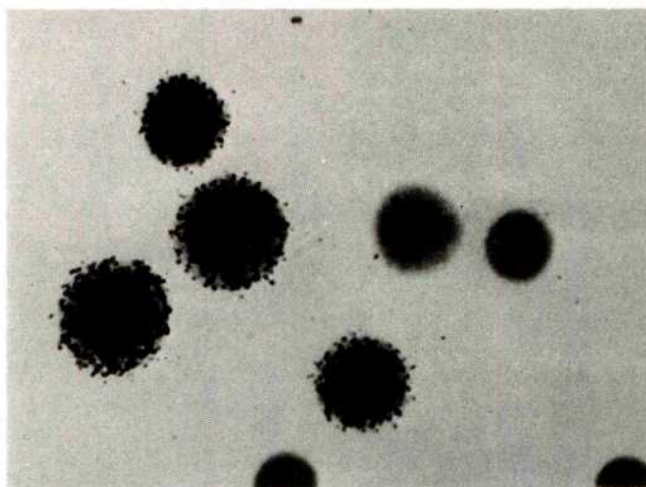


Fig. 1 Autoradiographs of dry smears of human peripheral blood lymphocytes cultured for 48 h in the presence of PHA. Human peripheral blood lymphocytes were obtained from heparinised blood. Culture techniques were based on those described by Moorhead *et al.*⁵ and Hungerford⁶. PHA (reagent grade, Wellcome, UK, reconstituted) was used at a concentration of 0.1 ml per 10 ml culture medium. Cultures were maintained at 37 °C for 48 h. At 41 h, 5 μ Ci methyl-³H-thymidine (5 Ci mmol⁻¹) was added and after 7 h of labelling cells were collected, washed and resuspended in Hanks' balanced solution to a concentration of 1×10^6 ml⁻¹. This suspension, which contained more than 70% lymphoid cells, was split into two. One half was treated according to Moorhead *et al.*⁵ and Hungerford⁶. (Fixing with a mixture of methanol-acetic acid (3:1 v/v) and dry smears of the labelled lymphocytes were prepared. The slides were washed once with 5% Cl₃CCOOH, once with methanol and allowed to dry. The other half of the cultured lymphocytes was mixed with an equal volume of a 0.5% suspension of SRBC and after appropriate incubation (room temperature for 1 h) multilayer rosettes were formed spontaneously. These rosettes remain intact if they were reincubated at 37 °C for 60 min. A multilayer rosette was defined as a lymphocyte surrounded by at least 30 or more SRBC. From these rosettes thin smears were prepared. We used two kinds of control: one with lymphocytes without incubation⁴ and one with lymphocytes incubated for 48 h without PHA before rosetting. The percentage of rosette formation was the same for both, unless they were incubated for 60 min in an ice bath. Furthermore, the rosettes so formed contained only 5-10 SRBC. This observation is in agreement with the findings of Lay and Mendes⁴. The proportion of lymphocytes binding SRBC was found to be 10-15%, whereas Lay and Mendes⁴ have reported 20-40% and Bach *et al.*^{7,8} 0.4-2.6%. Cells were overlaid with a photographic emulsion (Kodak NTB2) and allowed to set for 3 d; after fixation they were counterstained with Giemsa.

the thin smears (multilayer rosettes) were prepared (for details, see Figs 1 and 2). Figure 1 shows that heavily labelled lymphocytes are present after a 48 h culture period. Up to 80% of the cells become blast-like in appearance and contain ³H-thymidine. Figure 2 shows the formation of multilayer rosettes as a result of incubating SRBC with ³H-labelled lymphocytes. Almost 70% of the lymphocytes formed rosettes, and the striking fact is the almost complete transfer of label from those lymphocytes to the SRBC (Fig. 2). Using human and rabbit erythrocytes instead of SRBC, no rosettes were formed at all, indicating that rosette formation does not depend on the presence of residual PHA on the surface of the blast.

To confirm that intracellular ³H-thymidine was in DNA the labelled cells were collected, taken up in a solution of sodium dodecyl sulphate (1.5%) in 0.01 M Tris buffer, pH 7.4, with 0.01 M EDTA, treated with NaOH at a final concentration of 0.3 M, precipitated with Cl₃CCOOH at a final concentration of 5% and collected on Millipore filters. These were washed twice with 5% Cl₃CCOOH, once with methanol, dried and counted in a scintillation counter. Acid-precipitable ³H-thymidine was determined. Furthermore, the nature of the label observed in SRBC after rosette formation was determined as follows. After culture

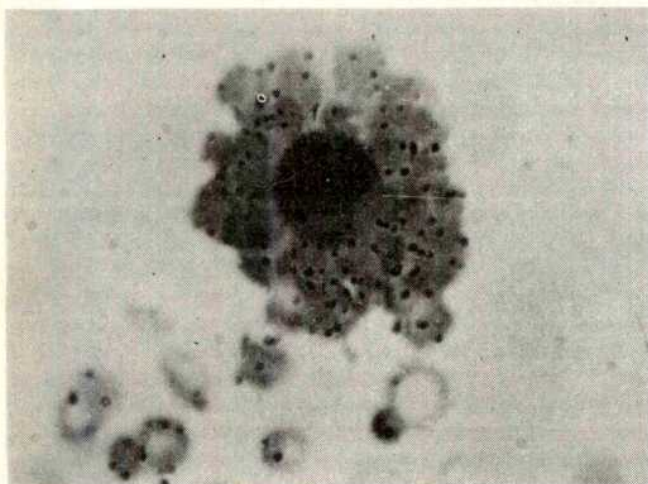


Fig. 2 An equal volume of lymphocytes ($1 \times 10^6 \text{ ml}^{-1}$) and 0.5% suspension of SRBC were mixed. The tubes containing the mixture were incubated at 37°C for 5 min, centrifuged for 5 min at $200g$, left at room temperature for 60 min, gently rocked to resuspend the cells in the pellet and thin smears were prepared. Autoradiographs were prepared as described in Fig. 1.

for 48 h duplicate tubes of labelled cultured lymphocytes were collected. On one set acid-precipitable ^3H -thymidine (c.p.m.) was determined as mentioned above; on the other set the cells were incubated with SRBC and rosettes were formed which were centrifuged at $500g$ for 10 min (supernatant I). The cell pellet was dissolved in distilled water and centrifuged at $5,000g$ for 10 min (supernatant II). In the cell pellet as well as in the two supernatants, acid-precipitable radioactivity was determined. That found in the labelled lymphocytes (see above) was identical to that in the cell pellet. The two supernatants contain almost zero acid-precipitable ^3H -thymidine radioactivity, indicating that acid-precipitable material is not present in the haemolysate, but bound to the erythrocyte stromata.

Human lymphocytes which had not incorporated ^3H -thymidine into their DNA, do not bind SRBC, although the thin smears were thoroughly scanned. This could mean that these cells have not been stimulated by PHA and are therefore unable to form rosettes. Furthermore, we have noted that PHA-stimulated lymphocytes lose their ability to form rosettes when fixed in a tube with a mixture of methanol-acetic acid (3:1 v/v). In this case lymphocytes were cultured as described previously, fixed in the tube and washed thoroughly in Hank's balanced solution. No rosettes were observed when we used lymphocytes so treated, indicating that cells must be viable to form rosettes.

Lymphocytes undergoing spontaneous transformation excrete DNA in a manner similar to lymphocytes stimulated with PHA and it is likely that this phenomenon is an intrinsic property of the lymphocyte rather than a special function induced by PHA. The observed presence of labelled DNA in the SRBC may reflect the transfer of ^3H -DNA between lymphocytes and SRBC. Although the explanation for the appearance of DNA in SRBC is obscure, a number of possibilities may be considered. Rosette formation is an interaction between a lymphoid cell and a heterologous erythrocyte which, although having some degree of specificity, is probably not immunological in nature. It would be difficult to admit that such a high proportion of peripheral blood lymphocytes (60–80%) have anti-SRBC antibodies on their membranes.

On the other hand the fact that the multilayer rosettes remained intact even at 37°C for 1 h or more is in agreement with the findings of Bach *et al.*^{7,8} for cluster formation between erythrocytes and lymphoid cells from immunised animals. A tentative hypothesis is to consider this phenomenon as a direct cell–cell interaction, which would depend

on the close apposition of large areas of the surfaces of the two cells in a manner impossible to achieve unless both cells were intact and possibly viable⁹. Finally, studies with a long term lymphocyte cell line¹⁰ have shown that about 0.5% of this DNA is associated with the cytoplasmic membrane. Whether these species of DNA are related to the DNA appearing in the SRBC is not known. Further studies on the functional significance of the findings reported here are now in progress.

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Are contact hypersensitivity cells cytotoxic?

THYMUS-DERIVED lymphocytes (T cells), which participate in many regulatory and effector functions of the immune system, are divided into distinct subpopulations. Thus, T cells causing cell-mediated cytotoxicity can be distinguished from those cooperating with bone marrow-derived lymphocytes (B cells) in the humoral response^{1–3}. Cytotoxic T cells also seem to be distinct from the cells responsible for the mixed lymphocyte and graft versus host reactions^{4,5}. Furthermore, T-cell subpopulations can interact with each other to augment various cell-mediated immune responses^{6–8}. We have investigated whether the T cells causing contact sensitivity belong to a different subset from those causing cell-mediated cytotoxicity. Our studies were based on two observations: first, that in mice contact sensitivity to the trinitrophenyl group (TNP) can be transferred adoptively by spleen or lymph node cells of sensitised animals; and, second, that cytotoxic T cells able to lyse TNP-coupled target cells can be induced *in vitro*^{11–13}. We have found that contact sensitivity cells fail to display cytotoxicity, while cytotoxic cells fail to transfer contact sensitivity. From this we conclude that these two functions are performed by distinct T cell subpopulations.

To induce contact sensitivity to TNP, C57BL/6 mice were sensitised twice by applying 0.1 ml 5% picryl chloride in ethanol to the clipped abdominal skin¹⁴. Five days later their ears were painted with 1% picryl chloride in olive oil and the increase in ear thickness determined after 24 h. Table 1 shows considerable ear swelling in mice sensitised twice, indicating significant contact sensitivity to TNP. After measurement the mice were killed and the draining lymph nodes (axillary and inguinal) were removed, minced, washed and suspended in RPMI-1640 (Microbiological Associates) containing 10% foetal calf serum. Aliquots of 5×10^7 cells were injected into normal C57BL/6 recipient mice, which were challenged immediately with 1% picryl chloride in olive oil applied to the ears. The lymph node cells from these sensitised mice transferred strong contact sensitivity, while the lymph node cells from normal mice did not.

Table 1 Transfer of contact sensitivity to TNP by lymph node cells and their inability to lyse TNP-coupled target cells

Contact sensitivity		Increment of ear thickness at 24 h in 10^{-3} cm \pm s.e.	
Test animals			
Donors: normal mice		7.0 \pm 0.8	
mice sensitised on day -6 and day -11		29.9 \pm 1.7	
Recipients: normal mice		5.0 \pm 0.2	
injected with 5×10^7 normal lymph node cells		6.1 \pm 1.5	
injected with 5×10^7 TNP-immune lymph node cells		15.8 \pm 1.1	
Cytotoxicity		% Cytotoxicity \pm s.e.	
Source of cells	Target cells	4 h	8 h
C57 B16 spleen sensitised to TNP-BALB/c (<i>in vitro</i>)	EL4	0	1.7 \pm 1.6
C57 B16 spleen sensitised to TNP-BALB/c (<i>in vitro</i>)	TNP-EL4	20.4 \pm 1.6	39.5 \pm 3.3
C57 B16 lymph node cells from TNP-C1 sensitised donor	EL4	0	0.3 \pm 0.2
C57 B16 lymph node cells from TNP-C1 sensitised donor	TNP-EL4	0	3.5 \pm 2.7

Donor mice: C57BL/6 mice were sensitised on days -12 and -6 with 100 μ l 5% picryl chloride and challenged on day -1 with 1% picryl chloride on both ears. Ear swelling was determined after 24 h. The mean increments in 10^{-3} cm \pm s.e. are given. Draining lymph nodes were removed and assayed for cell-mediated cytotoxicity at an attacker to target cell ratio of 100:1 on normal and TNP-coupled EL4 target cells¹². As a control, cytotoxic cells able to lyse TNP-EL4 targets were induced *in vitro* by culturing 10^7 C57BL/6 spleen cells with 5×10^4 TNP-coupled BALB/c spleen cells per ml (ref. 12). Spontaneous ^{51}Cr release was 3.2% h^{-1} for EL4 and 3.4% h^{-1} for TNP-EL4 targets. Recipient mice: 5×10^7 lymph node cells from normal and sensitised donors were injected into groups of five normal mice, which were challenged with 1% picryl chloride on their ears, to demonstrate their capacity to transfer contact sensitivity.

Percentage cytotoxicity calculated as:

$$\frac{(\text{experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})}{(\text{maximal } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})} \times 100$$

The same lymph node cell preparation was tested for cytotoxicity toward normal or TNP-coupled syngeneic EL4 lymphoma target cells. At an attacker to target cell ratio of 100:1 and within 8 h these lymph node cells were cytotoxic neither to EL4 cells nor to TNP-EL4 cells. By contrast, C57BL/6 spleen cells sensitised *in vitro* to TNP-coupled BALB/c spleen cells¹² killed TNP-EL4 very efficiently, but not EL4, demonstrating that the TNP-EL4 were suitable targets for cytotoxicity. The failure of sensitised lymph node cells to lyse TNP-EL4 cells could have one of three explanations: (1) cells cytotoxic to hapten-coupled target cells are not present in the population responsible for the specific transfer of contact sensitivity to TNP; (2) cytotoxic cells are present in the sensitised population but are inactive (for example, because of suppression); or (3) cytotoxic cells are present but their activity cannot be detected because the assay is not sufficiently sensitive.

To distinguish between these possibilities, C57BL/6 spleen cells were sensitised *in vitro* to TNP-coupled syngeneic spleen cells¹². Table 2 shows that these spleen cells exerted high cell-mediated cytotoxicity on TNP-EL4 targets but no cytotoxicity on EL4 target cells. Various doses of cytotoxic effector cells (5×10^4 – 5×10^7) were injected intravenously into normal C57BL/6 mice, which were immediately challenged with 1% picryl chloride in olive oil on the ears. Table 2 shows that no contact sensitivity to picryl chloride was transferred by any dose of the cytotoxic spleen cells. It seems unlikely that this inability of the cytotoxic spleen cells to transfer contact sensitivity is caused by culturing these cells, because *in vitro* sensitised cytotoxic spleen cells transfer cell-mediated immunity to mice (ref. 15 and our unpublished results). Moreover, *in vivo* sensitised lymph node cells able to transfer contact sensitivity to TNP do not lose this ability after 5 d in culture (our unpublished results). This experiment (Table 2) therefore rules out the third possibility, since cells sufficiently active to be detected by the cytotoxicity assay did not transfer contact sensitivity even at very low doses. The second possibility also seems to be excluded because the population of cells cytotoxic to TNP-EL4 was nonetheless inactive in the contact sensitivity reaction. The first possibility is thus the most likely, implying that cells able to transfer contact sensitivity to TNP can be found in the absence of cells able to cause cytotoxicity to TNP-coupled target cells. Since both cytotoxicity to TNP-EL4 and contact sensitivity to hapten are functions of

T cells^{9–12}, they seem to be properties of two T-cell subpopulations.

Our results seem to conflict with experiments¹⁸ in which lymph node cells of CBA mice sensitised to either oxazolone or picryl chloride were able to lyse the allogeneic DBA/2 mastocytoma P815, provided phytohaemagglutinin was present¹⁸. This suggests that potentially cytotoxic cells are present in the lymph nodes of contact sensitised mice that are absent in normal mice; it does not necessarily mean, however, that the contact sensitivity cells themselves are cytotoxic, since the cytotoxicity could not be demonstrated to be target cell specific¹⁸. Arguing against the

Table 2 Inability of cytotoxic spleen cells to transfer contact sensitivity to TNP

Contact sensitivity		Increment in ear thickness at 24 h in 10^{-3} cm \pm s.e.	
Test animals			
Normal mice		3.6 \pm 0.4	
Mice sensitised on day -6		13.0 \pm 1.3	
Recipients of 5×10^7 non-immune spleen cells		4.8 \pm 1.5	
5×10^7 immune spleen cells		4.9 \pm 1.4	
5×10^6 non-immune spleen cells		3.8 \pm 1.1	
5×10^6 immune spleen cells		4.2 \pm 1.6	
5×10^5 non-immune spleen cells		3.9 \pm 1.7	
5×10^5 immune spleen cells		3.5 \pm 0.9	
5×10^4 non-immune spleen cells		5.0 \pm 2.4	
5×10^4 immune spleen cells		4.3 \pm 1.2	
Cytotoxicity		% Cytotoxicity \pm s.e.	
Source of cells	Target cells	4 h	8 h
C57 B16 spleen sensitised to TNP-C57 B16 spleen	EL4	0	0
C57 B16 spleen sensitised to TNP-C57 B16 spleen	TNP-EL4	16.4 \pm 1.4	48.1 \pm 3.7

C57BL/6 spleen cells (10^7 ml^{-1}) were sensitised *in vitro* to TNP-C57BL/6 spleen cells ($5 \times 10^5 \text{ ml}^{-1}$)¹². After 5 d cultures were collected and cytotoxic activity assayed on EL4 and TNP-EL4 target cells at an attacker to target cell ratio of 100:1. Spontaneous ^{51}Cr release was 2.9% h^{-1} for EL4 and 3.1% h^{-1} for TNP-EL4 targets. Spleen cells of cultures incubated with or without antigen were injected in various doses into groups of five normal recipients which were challenged with 1% picryl chloride. As controls, normal mice and mice presensitised once with picryl chloride on day -6 were included.

identity of contact sensitivity and cytotoxic cells is the finding that resensitisation of mice decreases the cytotoxic activity in the lymph nodes, while it increases their potential to transfer contact sensitivity (ref. 16 and our unpublished results).

It could be argued that the hypersensitivity cells are cytotoxic but do not lyse the TNP-EL4 target cell because they do not recognise the TNP group, but rather some larger moiety, including the determinant to which TNP is coupled. Evidence for this possibility has been presented with respect to the guinea pig^{17,18}. Recent experiments, however, have shown that cytotoxic mouse spleen cells, able to lyse the TNP-EL4, can recognise both the TNP group and TNP-modified self antigens¹¹⁻¹³ so that the failure of such cytotoxic cells to demonstrate contact sensitivity *in vivo* cannot be ascribed to inability to recognise the TNP group.

On the assumption that antigen recognition in the contact sensitivity reaction is similar to that in the cytotoxic reaction, our conclusion is that these reactions are performed by two distinct T-cell subpopulations.

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Appearance of foetal antigens in somatic cells after interaction with heterologous sperm

GENETIC traits may be transferred to tissue cultured mammalian cells by interaction with viruses^{1,2}, nucleic acids^{3,4}, and cellular organelles such as metaphase chromosomes⁵⁻⁷, and by other cells⁸. Recent findings⁹ indicate that incubation of mouse spermatozoa with Chinese hamster bone marrow fibroblasts (CLM) results in uptake of sperm, morphological alterations, and, at a frequency of about 0.01%, subsequent mouse foetal antigen expression in the heterologous fibroblast progeny. To learn whether the efficiency of these processes can be enhanced, combinations of several species of sperm and target cells were studied in orienting experiments¹⁰.

The interaction of rat spermatozoa with Chinese hamster lung fibroblasts (DON) in tissue culture led to a higher yield of altered cells than was observed in the CLM-mouse sperm system. For collection of spermatozoa, the vasa deferentia were excised aseptically from adult Wistar strain rats, placed in Brackett's solution¹¹ plus 5% foetal bovine serum (FBS), and the living sperm were gently extruded into the medium without mincing the vas deferens. The sperm were separated from any remaining tissue clumps and washed by brief low speed centrifugation in this medium and adjusted to a concentration of 3×10^7 sperm

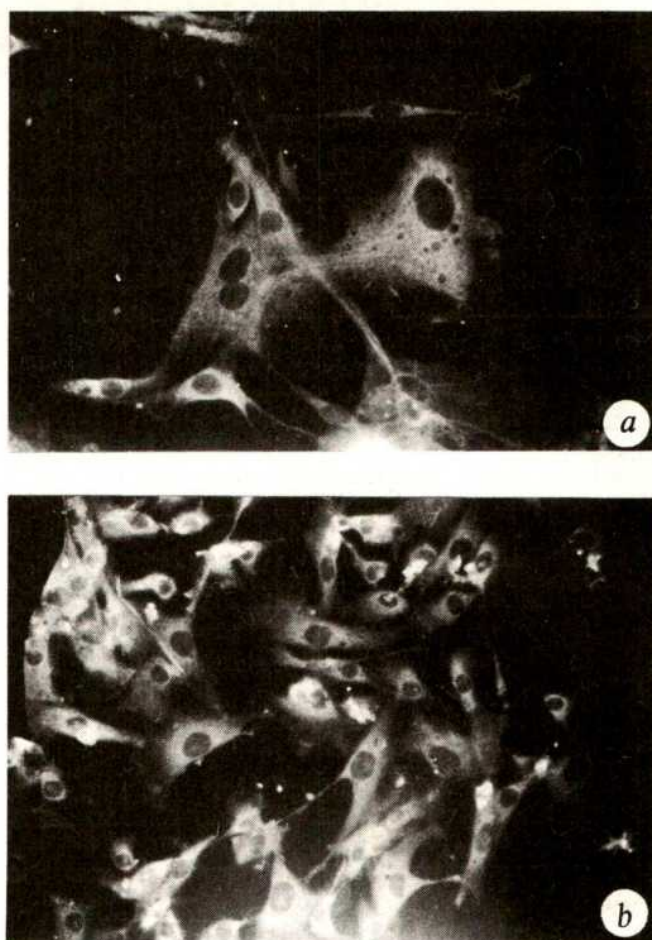


Fig. 1 Positive indirect immunofluorescence test for rat foetal antigens; ultraviolet light. *a*, Methanol-fixed DON Chinese hamster cells (from adult lung; American Type Culture Collection) 8 d after exposure to rat spermatozoa ($\times 248$). *b*, Methanol-fixed colony of DON cells 48 d after exposure to rat spermatozoa ($\times 157$). Cells were examined by the indirect immunofluorescence technique¹² by treating with Chinese hamster tissue-absorbed rabbit antiserum prepared against lyophilised skin-free trunk extracts of 17-21-d rat foetuses. After washing, the cultured cells were then stained with fluorescein-conjugated goat anti-rabbit IgG which had previously been absorbed with both Chinese hamster tissue extract and rat liver extract.

ml^{-1} . A suspension of 8×10^5 DON cells in 1.6 ml of Ham's F12 medium which contained 2% heat-inactivated FBS was mixed with 0.4 ml of the above sperm suspension. The ratio of sperm to cells was thus 15:1. After 2 h at 37 °C, 0.4 ml aliquots of the mixed cells were pipetted into 60-mm Petri dishes which contained microscope cover slips, and 4.6 ml of F12 medium plus 20% heat-inactivated FBS was added to each. Incubation was at 37 °C in a moist atmosphere of 5% CO_2 in air, and coverslips were withdrawn for examination after various times³. Control cultures of DON cells were incubated in parallel in the same final medium. Cell morphology was studied microscopically after fixation of monolayers with methanol and staining with Giemsa. By 48 h after admixture, about 50-55% of the DON cells seemed to have been penetrated by at least one spermatozoon. Colcemide-treated cultures were used to study metaphase chromosomes at various times after the first day of admixture.

Control DON cultures maximally showed a 3% incidence of binucleate cells and an occasional cell with three or four nuclei. In contrast, the fibroblasts in the mixed cultures showed a higher proportion of multinucleates beginning about 24 h after exposure to sperm, and at 96 h 14% were multinucleate. Although binucleation was the major morphological change in the 96-h mixed cultures, many of the

sperm-treated cells had four to six nuclei per cell. The proportion of cells in sperm-treated cultures with an approximately tetraploid number of chromosomes was also higher than in control populations. The modal chromosome number of the DON cells in control cultures was 22. Two days after exposure to rat sperm, 8% of the treated cells contained 40 or more chromosomes. Parallel control cultures at this stage had a 2% frequency of tetraploid cells and these possessed 44 chromosomes. The incidence of near tetraploids (cells with 40–50 chromosomes) 3 d after exposure to sperm had increased to 14%. Control values of tetraploid cells never exceeded 3% during the course of this study. Statistically, the increase over control values seen in rat sperm-treated cultures was highly significant, $P < 0.01$.

Progeny Chinese hamster cells at various times after interaction with spermatozoa were examined for the presence of rat antigen by the indirect immunofluorescence technique¹² using antiserum prepared in the rabbit against lyophilised preparations of saline extracts¹³ of days 17–21 Wistar rat foetal tissue, and staining with fluorescein-conjugated goat anti-rabbit IgG. The rabbit antiserum against rat foetal tissue extracts and the goat anti-rabbit IgG were absorbed with lyophilised extracts and whole tissue homogenates of adult Chinese hamster organs. The conjugated goat anti-rabbit IgG was further absorbed with lyophilised extracts of rat liver. At day 8, but not before that, approximately 0.5–1.0% of the cells examined in the ultraviolet showed positive cytoplasmic immunofluorescence (Fig. 1a). Of the positive cells, 18% had more than one nucleus, with binucleates predominating in this group; about 10% of the positive cells had a vacuolated cytoplasm. At days 14 and 36 after interaction with sperm, the proportion of progeny cells which exhibited positive cytoplasmic immunofluorescence increased to about 3% and 4% respectively. At day 39, cells were lightly seeded on coverslips to obtain individual colonies; after 9 d, all the cells in 1–2% of these colonies showed positive immunofluorescence (Fig. 1b).

Control DON cells and the washed rat spermatozoal preparations used were consistently negative with both immune and pre-immunisation rabbit serum; sperm-treated DON cells were negative throughout the 48-d period when tested with preimmune serum. Cultured Fisher rat embryo cells were positive with immune serum, but cultured adult Gunn rat liver cells¹⁴ and Morris hepatoma cells were negative. Rabbit antiserum against whole adult rat serum, after absorption with Chinese hamster tissue as above, gave positive immunofluorescence tests with the rat liver cells, but the tests were negative when applied to DON cells before and after mixture with rat sperm. Contaminating rat somatic cells in the sperm preparation would have been revealed in this test.

Experiments are under way to isolate and characterise the component(s) responsible for the immunofluorescence in individual positive clones, and to learn whether tumorigenicity can be demonstrated. In previous studies^{3,4}, synthesis of mouse antigens continued in CLM cells for at least a year of growth after interaction with mouse tumour cells or with DNA isolated from the latter; oncogenic potential was also acquired.

Although rigorous evidence that transcription and translation of rat sperm genetic material occurred in the DON population is not yet provided in these ongoing studies, it is unlikely that the results reported could have been due to rat somatic cells which had contaminated the sperm. Karyotype analyses never revealed the presence of rat chromosomes in hundreds of metaphase plates examined after coculture with the rat sperm. Immunofluorescence tests at times earlier than 8 d were consistently negative for rat foetal antigen. Furthermore, somatic cells of rat origin did not propagate when the sperm preparations themselves were subjected to the tissue culture conditions

used. We conclude that the intense cytoplasmic fluorescence observed after the interaction of rat sperm and DON cells was a result not of the presence of rat somatic cells or sperm proteinaceous material, but of the synthesis of rat foetal protein by the hamster cells.

The immunofluorescence data indicate that rat protein, most probably of foetal nature, persisted for at least 40–50 generations in Chinese hamster cells after mixture with rat sperm. Some proteins regarded as characteristic of the foetal stage of ontogeny are known to reappear in the adult just before the onset of and during malignant growth^{15,16}.

The data presented provide additional evidence that mammalian spermatozoa are capable of penetrating somatic cells *in vitro* and of affecting morphology and the normal process of mitosis. Independent confirmation of such penetration has appeared^{17,18}. The results in the present report suggest that sperm can act as vectors for the transfer and subsequent expression of genetic information in heterologous somatic cell systems. Whether the results are a consequence of a fertilisation-type reaction or are due to the presence of a recently discovered RNase-sensitive endogenous DNA-synthesising complex within sperm heads¹⁹, or to a still unknown mechanism is not yet clear. It is hoped that use of the sperm-somatic cell system will provide a further understanding of the significance of foetal proteins in oncogenesis^{20,21}.

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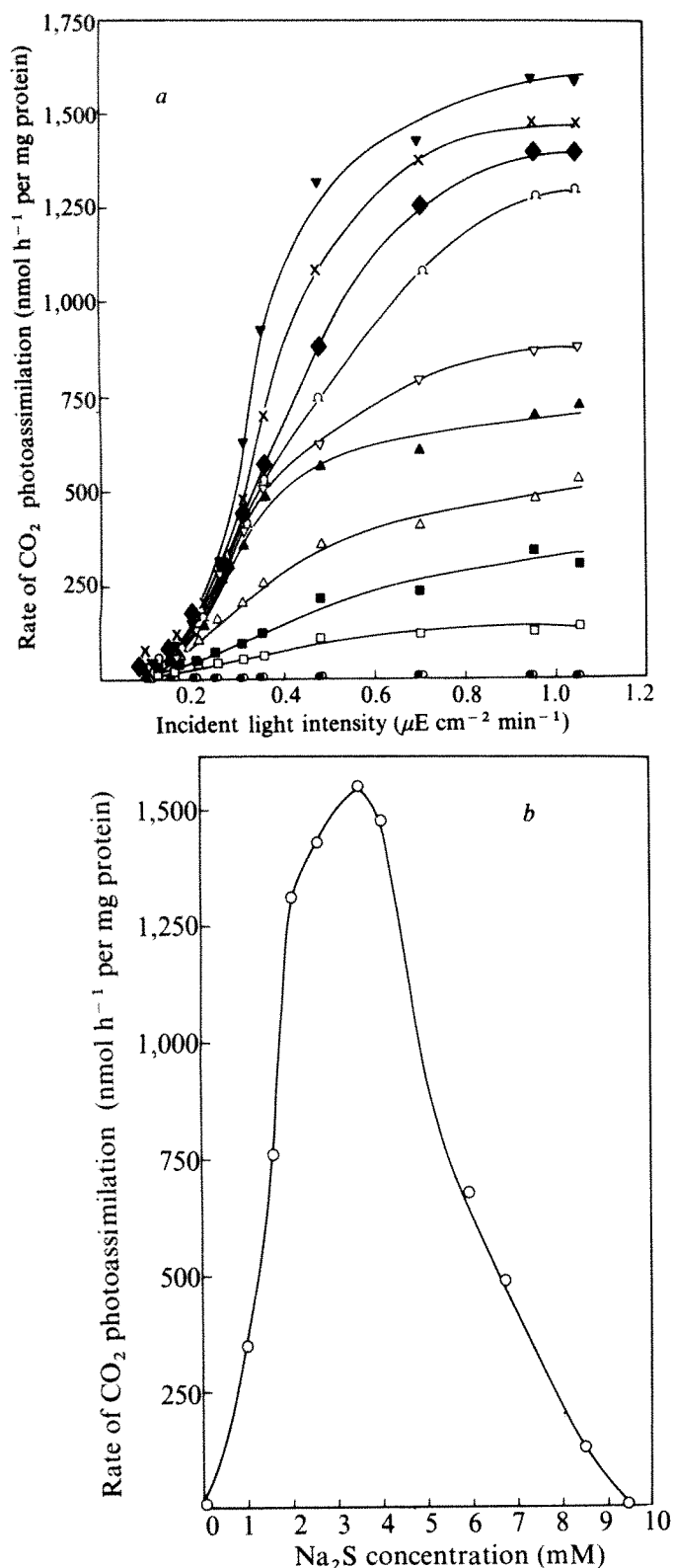
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Sulphide-dependent anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*

PROFOUND differences in structure and function separate the prokaryotic cyanobacteria (blue-green algae) from eukaryotic algae and plants, whereas oxygenic photosynthesis is considered to distinguish the cyanobacteria and eukaryotic plants from the



other phototrophic bacteria. The prevailing concepts on the cyanobacteria emphasise their prokaryotic nature and oxygenic photosynthesis. This combination of properties, among others, led to the suggestion that the cyanobacteria represent a group of possible progenitors of chloroplasts¹. While cyanobacteria typically exhibit oxygenic photosynthesis with two photosystems using electrons from water, it has been demonstrated that photosystem I of certain cyanobacteria can function *in vivo* independently in supporting virus production², heterocyst functions³ and even photoheterotrophic growth⁴. We have demonstrated anoxygenic photosynthesis in a cyanobacterium *Oscillatoria limnetica*, also capable of oxygenic photosynthesis,

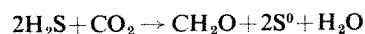
Fig. 1 *a*, CO₂ photoassimilation by *Oscillatoria limnetica* in the presence of DCMU at different Na₂S concentrations and intensities of 704-nm wavelength light. Twice-washed, logarithmic phase cells were suspended in the growth medium (15 μg cell protein per ml) at various Na₂S concentrations with DCMU (5 μM) in 5-ml stoppered vials and preincubated for 2 h at 35 °C in light provided by a 60-W tungsten lamp (incident intensity of 2×10^4 erg cm⁻² s⁻¹). NaH¹⁴CO₃ was introduced to a final specific activity of 1.50 μCi μmol⁻¹, and the suspension incubated for 5 min under the actinic light provided by a Prado Universal-Leitz projector and filtered through a Corning HR 2-60 filter and then Schott Mainz sharp cutoff interference filter blocked to infinity peaking at 704 nm (11-nm half bandwidth). The incident intensity of the actinic light, varied by using a powerstat, was measured by a Yellow Springs Instrument Radiometer model 65. The cell suspension was filtered on glass filter paper (Whatman GF/C), washed with 40 ml 0.6 M trichloroacetic acid (4 °C), and cell radioactivity was counted by a gas-flow counter. Filters were washed with 5 ml of absolute ethanol to remove elemental sulphur, and protein was determined⁹. Various Na₂S concentrations in mM, were determined (methylene blue photometric method¹⁰) when the radioactivity was added. Concentrations (mM): ▽, 3.5; ×, 4.0; ◆, 2.8; ○, 2.0; △, 1.6; ▲, 5.9; □, 6.7; ■, 1.0; □, 8.5; ●, 0.0; ○, 9.5. *b*, Plot of photoassimilation rate (from *a*) at the saturation light intensity (1.0 μerg cm⁻² min⁻¹) as function of Na₂S concentration.

which was isolated from the anaerobic H₂S rich hypolimnion layer of the monomictic, hypersaline Solar Lake located on the desert margin of the Gulf of Elat, Israel⁵. During winter stratification, this layer is located below two plates of phototrophic sulphur bacteria (*Chromatium violescens* and *Prosthecochloris* sp.), and exhibits a dense cyanobacterial bloom with very high rates of primary production. We report here evidence for the photosystem I-driven CO₂ photoassimilation in *O. limnetica* with H₂S serving as sole electron donor through oxidation to elemental sulphur.

O. limnetica was grown in pure culture in a mineral medium prepared in Solar Lake water (96.7‰ chlorinity; 174.1‰ salinity), containing the major elements (g l⁻¹): KH₂PO₄, 0.33; NH₄Cl, 0.33; MgCl₂·6H₂O, 0.33; KCl, 0.33; NaHCO₃, 1.50; Na₂S·9H₂O, 0.075; vitamin B₁₂, 10⁻⁵; as well as SL 4 trace elements⁶; final pH was adjusted to 6.8 with HCl. Cultures were grown in completely filled glass-stoppered bottles, illuminated continuously by white fluorescent lamps (4,300 K, 20 W, incident light intensity 8×10^3 erg cm⁻² s⁻¹) at 35 °C.

The ability of the cyanobacterium to photoassimilate CO₂ in reactions driven by photosystem I alone and using Na₂S was demonstrated in the presence of DCMU under actinic light of 704-nm wavelength (Fig. 1*a*); no photoassimilation was observed in the absence of sulphide or light. Although the detailed mechanism of the sulphide oxidation is not known, the photosystem I-driven reaction and use of Na₂S are similar to that observed in many phototrophic sulphur bacteria. This photoassimilation is saturated by 704-nm wavelength light at an incident intensity of about 1.0 μerg cm⁻² min⁻¹. The plot of the photoassimilation rate at light saturation as a function of sulphide concentration shows an optimum around 3–4 mM Na₂S (Fig. 1*b*).

The fate of the sulphide, oxidised during the Na₂S-photosystem I-driven CO₂ photoassimilation in *O. limnetica* was determined from the distribution of the radioactive label in different sulphur fractions of the culture incubated with Na₂³⁵S in the presence of DCMU (Fig. 2). The decrease in ³⁵S²⁻ is accompanied by an almost equivalent increase in ³⁵S⁰. The small increase in ³⁵S₂O₃²⁻ and ³⁵SO₄²⁻ together are accounted for by chemical sulphide oxidation (as observed in the cell-free control). It seems that *O. limnetica* photo-oxidises S²⁻ quantitatively to S⁰. The rate of Na₂S oxidation in this experiment in the 10–25-h interval was 3.7 μmol Na₂S per mg protein per h; the rate of CO₂ uptake measured in identical conditions was 1.8 μmol CO₂ per mg protein per h. The stoichiometric relationship between CO₂ photoassimilation and H₂S oxidation derived, suggests the following equation:



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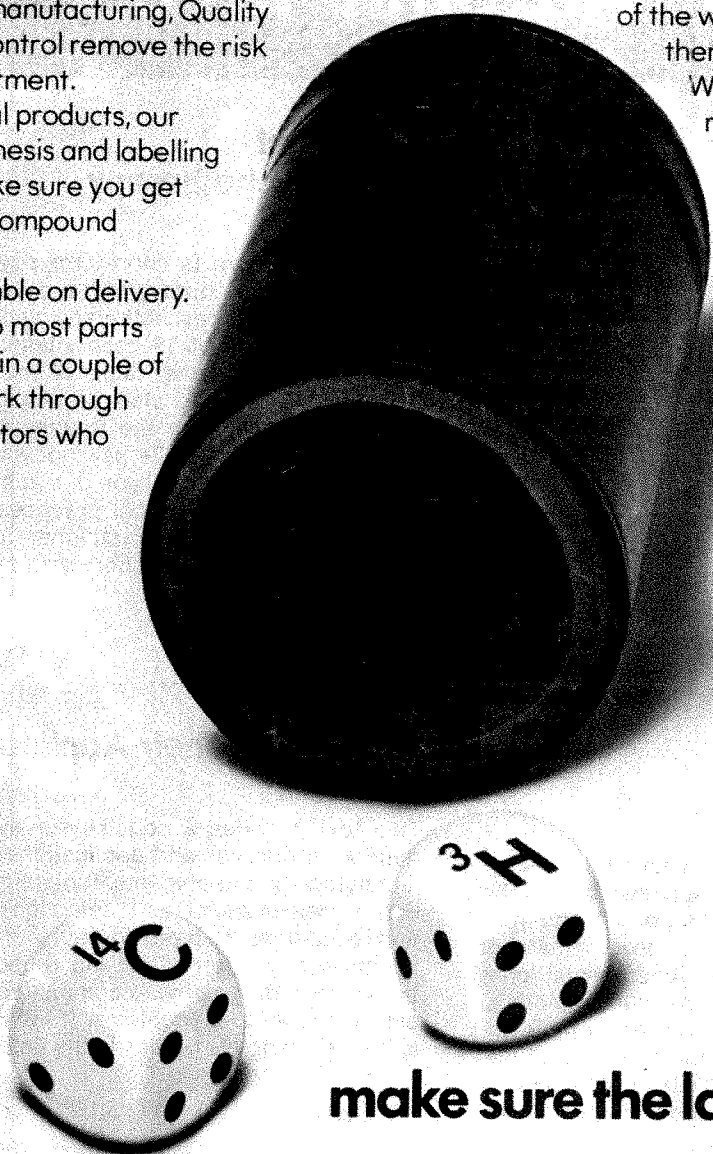
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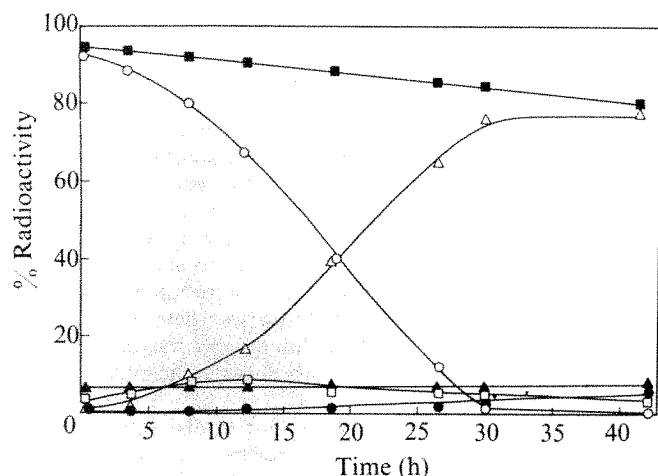


Fig. 2 Sulphide photo-oxidation by *Oscillatoria limnetica*. Cells, as described in Fig. 1, were suspended (25 µg cell protein per ml) in growth medium and flushed thoroughly with oxygen-free N₂. Cells were preincubated in the dark at 35 °C for 2 h, after which Na₂³⁵S was added anaerobically to a final specific activity of 0.1 µCi µmol⁻¹ and then incubated at 35 °C in continuous light provided by a 40-W tungsten lamp at a distance of 40 cm. Dark control and blank (without cyanobacteria) for chemical sulphide oxidation were run in parallel; in both cases sulphide oxidation did not exceed 12% throughout the experiment. Samples (5 ml) were filtered in a Swinnex filter holder through a 0.45-µm (average pore size) membrane filter directly into 2 ml of 0.11 M CdCl₂; filters were washed once with 10 ml of oxygen-free water, which was added to the filtrate (soluble S²⁻ fraction). The filter retaining both the particulate S⁰ and organic S fractions was homogenised in distilled water (5 ml) and transferred to a scintillation vial. These latter fractions were separated in another 5 ml sample of the experimental suspension. S⁰ and pigments were extracted by filtering the suspension and treating the glass filter with 5 ml of absolute ethanol, and were determined photometrically in the filtrate¹⁰. The filter retaining the organic S fraction was homogenised and transferred to a scintillation vial. The soluble S²⁻ fraction together with 0.1 nmol CdS carrier was transferred to an apparatus for anaerobic distillation¹¹. After flushing the apparatus with N₂, 10 ml of oxygen-free 6 N HCl was added. Sulphide was distilled into 10 ml of 0.1 M Zn acetate at room temperature with N₂ as carrier gas; 5 ml of the suspension of the ZnS produced was transferred to a scintillation vial for counting the ³⁵S²⁻ fraction. To precipitate sulphate, 5 ml of 2 M BaCl₂ was added to the undistilled remaining solution, diluted to 50 ml with water at room temperature. A 5-ml sample was transferred to a scintillation vial to determine both ³⁵SO₄²⁻ (as Ba³⁵SO₄) and dissolved ³⁵S₂O₃²⁻; the radioactivity in the remaining filtrate represented only the S₂O₃²⁻ fraction, although this was not demonstrated chemically. Insta-gel scintillation liquid (5 ml) was added to all the vials for counting in a Tri-carb scintillation counter. Radioactivity determinations of the various sulphur fractions are expressed as percentage of total sulphur at each sampling time. Experimental variation was no more than ±5%. ■, Blank H₂S; △, S⁰; ○, H₂S; ▲, S₂O₃²⁻; □, SO₄²⁻; ●, Organic S.

Elemental sulphur excreted from the cells was observed as typical refractile granules adhering to the cyanobacterial filaments (Fig. 3) or in the medium. The photosynthetic sulphur oxidation of Na₂S is similar in this respect to that of the Chlorobiaceae and some Chromatiaceae. In addition to the anoxygenic photosynthesis described here, this *O. limnetica* strain is capable of oxygenic photosynthesis and can readily shift to growth with either type of photosynthesis. The special physiological capacities of this strain represent an ecological advantage in an ecosystem such as the Solar Lake with an annual limnological cycle in which oxygenated conditions during holomixis alternate with high sulphide concentrations during stratification. *O. limnetica* is distributed throughout the lake at holomixis when the phototrophic bacteria disappear and thrives during stratification in the bottom layers, where the sulphide content is extremely high (40 mg H₂S per l) and prohibitive for the growth of the two other phototrophic bacteria.

The potency for using H₂ and other reducing compounds as electron donors for photoreduction of CO₂ has been demonstrated in special experimental conditions in certain oxygenic phototrophic organisms⁷, but growth was not supported. Attempts to grow other cyanobacteria in anaerobic conditions utilising H₂S as sole electron donor demonstrated that photosystem II is required⁸. *O. limnetica* represents the first case of an oxygenic phototroph that can grow anaerobically on H₂S. The significance in the evolution of oxygenic photosynthesis of a prokaryotic organism with both types of photosynthesis is highly suggestive. This strain also offers new possibilities for isolating photosystem II mutants.

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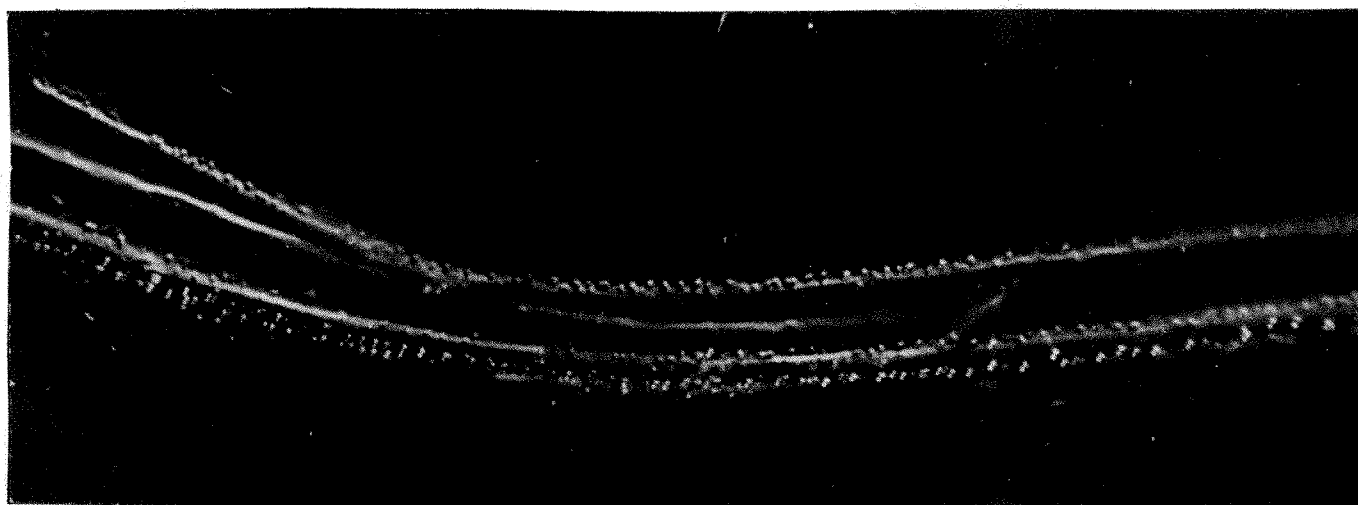


Fig. 3 *Oscillatoria limnetica* filament grown in anoxygenic conditions. Refractile sulphur granules are seen adhering to filaments. Nomarski interference contrast (×2,558).

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Light inhibits the labelling of gangliosides in chicken retina

WE have reported that the labelling of brain gangliosides is more extensive in chickens given labelled glucosamine and subsequently exposed to light than in other chickens kept in the dark¹. The opposite result was obtained with rat brain². These effects parallel the influence of light on the general activity of the animals, and we could not decide whether the cause was a direct or indirect stimulus on the labelling process. Similar studies with the retina may help to solve this problem, for the retina is stimulated directly by light, and it is possible, by occluding one eye, to measure labelling in different conditions of illumination in one animal. We have now done such experiments and found that the labelling of retinal gangliosides varies in response to light: these changes are not attributable to systemic effects.

Labelling of retinal gangliosides in chickens maintained in the dark was greater than in those exposed to light (Table 1, experiments 1 and 2). Results were similar when one eye was occluded (experiment 4). In the latter case the difference was smaller and so the possibility remained that in experiments 1 and 2, a general effect was added to that of the incident light. But a substantial direct inhibition by light of retinal ganglioside labelling seems to be proved by our data.

Values for ganglioside labelling given in experiments 1 and 2 were normalised by the radioactivity of the acid-soluble fraction, but these ratios do not reveal the interesting fact that there was no difference between the acid-soluble fractions from the two groups of animals. The radioactivity of the acid-soluble fraction is shown in experiments 1a and 2a of Table 1. These results suggest that light did not affect the permeability to

labelled glucosamine or its derivatives, unless there was some effect in very limited areas of the retina.

The difference in the labelling of the sialyl moiety was at least partially responsible for the change in the gangliosides (Table 1, experiment 3). On the other hand, no individual ganglioside could be singled out as being specifically affected by light and this was investigated by chromatography. Chickens kept in the light or dark received, respectively, injections of ³H- or ¹⁴C-glucosamine or vice versa. Two hours later they were decapitated and the retinae pooled and minced. The gangliosides obtained from this pool were run on thin-layer chromatography and the ³H/¹⁴C ratio was determined every 0.5 cm of the chromatogram. In seven experiments we could not identify a zone with a ratio of labelling significantly different from the others.

There have been reports of the hyperpolarisation of the photoreceptor membranes in the vertebrate retina exposed to light, compared with that in the dark (for a review see ref. 3). It has been speculated that this hyperpolarisation interrupts the secretion of neurotransmitter from the synaptic endings⁴. For gangliosides, the precise step inhibited by light is unknown, but their participation in a presynaptic phenomenon is suggested by the high affinity of gangliosides for toxins of the presynaptic effect, such as tetanus and botulinum toxins^{5,6}.

In chickens another striking observation is that whereas exposure to light inhibited the labelling of gangliosides in the retina, it stimulated labelling in the telencephalon¹ the possibility that inhibitory events lead to decreased ganglioside labelling in the chicken retina exposed to light and that excitatory events lead to an overall increase in brain ganglioside labelling is worthy of investigation. The effect seen in the rat brain², which is opposite to that observed in chicken brain, requires explanation.

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Table 1 Labelling of gangliosides, gangliosidic sialic acid and acid-soluble fraction of retinae from chickens in the light or in the dark

Experiment	Time of exposure (h)	No. of pairs	Chickens in		Difference (%)	P
			Light	Dark		
c.p.m. gangliosides per mg protein $\times 10^5$						
c.p.m. acid-soluble fraction per mg protein						
1	0.5	10	932 \pm 100	1,384 \pm 200	50.7 \pm 12	< 0.01
2	2	14	3,160 \pm 440	4,310 \pm 560	35.7 \pm 7	< 0.001
c.p.m. sialic acid per nmol sialic acid $\times 10^5$						
c.p.m. acid-soluble fraction per mg protein						
3	2	9	72 \pm 6	83 \pm 5	16.9 \pm 4	< 0.01
c.p.m. acid-soluble fraction per mg protein						
1a	0.5	10	4,465 \pm 360	3,993 \pm 395	-8.0 \pm 8	NS
2a	2	14	5,147 \pm 538	4,681 \pm 514	-5.1 \pm 7	NS
Chickens with one eye occluded						
		No. of chickens	Exposed eye	Occluded eye		
c.p.m. gangliosides per mg protein						
4	1	18	141 \pm 16	160 \pm 15	18.5 \pm 5	< 0.01

For experiments 1, 2 and 3, male Warren chickens were subjected, from hatching to the eighth day, to the conditions described previously¹, then maintained in the dark for 2 d and matched in pairs according to weight. After subcutaneous injections of 6-³H-glucosamine (specific activity 7.3 Ci mmol^{−1}; New England Nuclear, 0.7 μCi per g body weight), one animal of a pair remained in the dark while the other was exposed to 1,000 lx; chickens were decapitated 0.5 or 2 h later. For experiment 4, chickens were treated up to the tenth day as in the previous experiments; then they had one eye patched with black tape, injected with the radiochemical, exposed to light for 1 h and decapitated. Retinae were minced in water and gangliosides isolated by the trichloroacetic acid-phosphotungstic acid method⁷. For sialic acid determinations, gangliosides were hydrolysed in 0.1 N H₂SO₄ for 1 h at 80 °C and the hydrolysate was dialysed against water. Dialysed sialic acid was purified through Dowex I and determined by the thiobarbituric acid method⁸. Values given are means ± s.e.m.; the significance of the percentage differences was calculated by Student's *t* test for correlated data. Differences per cent are the means ± s.e.m. of individual differences for each matched pair. Values for retinae in light were considered 100%.

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Influence of antidiuretic hormone on release of lysosomal hydrolase at mucosal surface of epithelial cells from urinary bladder

THE permeability change induced by antidiuretic hormone (ADH) in amphibian urinary bladder is limited to the apical membrane of epithelial cells rich in intracellular secretion granules¹. These granules, which may correspond to lysosomes², are observed to migrate to and fuse with the apical membrane of toad bladder epithelia treated with oxytocin or cyclic AMP³. We have extended these observations and found that ADH elicits a marked, time-dependent increment in the release of lysosomal hydrolases into the extracellular medium of epithelial cells isolated from amphibian bladder. This effect is abolished by specific antiserum to ADH and reduced by preincubation of cells with cortisol. Additional experiments with intact bladder verify that the hydrolases so released occur predominantly at the mucosal surface. Moreover, cortisol antagonises the enhancement of transepithelial water transport and membrane conductance attributable to ADH action.

Epithelial cells were isolated from the urinary bladders of *Rana catesbeiana* (Mogul-Ed Corp., Oshkosh, Wisconsin) by procedures described previously^{4,5}. These cells were maintained at 22 °C and suspended in Ringer solution constituted as follows: 104.5 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, and buffered at pH 7.4 with 2.125 mM Na₂HPO₄/0.375 mM NaH₂PO₄. Aliquots (2 ml; approximately 3 × 10⁶ cells) were incubated in the presence or absence of hormone for selected times in a Dubnoff shaking incubator. The epithelial cells were then sedimented by centrifugation at 400g for 5 min at +15 °C in a Sorvall RC2-B centrifuge. Sedimented cells were solubilised in 0.1 N NaOH:0.1% (v/v) Triton X-100 (Rohm and Haas) at 37 °C (24 h) and analysed for protein by the method of Lowry *et al.*⁶. The cell-free incubation medium was centrifuged further for 1 h at 105,000g in a Beckman-Spinco Model L preparative ultracentrifuge. Activities of representative lysosomal enzymes were determined on aliquots of the resultant particle-free supernatant fractions. Total enzyme activity per min in each experiment was then expressed relative to mg epithelial cell protein.

Cathepsin B1 (EC 3.4.22.1) activity was determined essentially

as described by Szego⁷. Fluorescence of the reaction product of cathepsin-mediated hydrolysis of the substrate, carbobenzyloxy-Ala-Arg-Arg-4-methoxy-β-naphthylamide (provided by Dr R. E. Smith), was measured at the emission peak of 410 nm on an Aminco-Bowman spectrophotofluorometer, using an activation beam of 292 nm; 4-methoxy-β-naphthylamine standards (provided by Dr Edward Smithwick, Jr) were analysed concomitantly. β-Glucuronidase (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31) activity was determined at pH 4.5 by the method of Fishman⁷ as modified by Musa *et al.*⁸, using the substrate, phenolphthalein β-D-monoglucosiduronic acid (Sigma) at 37 °C. Acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) activity was determined in the presence of 0.1 M sodium citrate buffer, pH 5.2, by the fluorometric method of Campbell and Moss⁹ as modified by Szego *et al.*¹⁰. Fluorescence of the product, α-naphthol, resulting from hydrolysis of the substrate, α-naphthylphosphate, was measured at the emission peak of 510 nm with an activation wavelength of 335 nm.

Epithelial cells in the several experiments were exposed to ADH (arginine vasopressin, Sigma), arginine vasopressin antiserum (provided by Drs A. A. Rosenbloom and D. A. Fisher), angiotensin II (Sigma), cortisol (free alcohol, Sharp and Dohme, West Point, Pennsylvania) or combinations of these agents. Cell viability in the presence and absence of these test reagents was evaluated by dye exclusion methods as described previously⁵. At least 95% of all cells used here excluded the dye, Trypan blue, at a final concentration of 0.05% in Ringer solution.

Determinations of activities of characteristic lysosomal enzymes released from epithelial cells incubated with and without ADH are presented in Table 1. Incubation in the presence of 1 mU ADH ml⁻¹ for 7.5 min resulted in statistically significant increases in the activities of acid phosphatase, β-glucuronidase and cathepsin B1, with mean increases of 66%, 24% and 167% over controls, respectively. Cells incubated with 1 mU ADH ml⁻¹ for 15 min responded in a similar manner. Acid phosphatase, β-glucuronidase and cathepsin activities rose by 37%, 15% and 133% over the values in controls. Although cells incubated with a higher concentration of hormone, 100 mU ADH ml⁻¹, for 15 min released a significantly greater activity of β-glucuronidase (*P* = 0.05), activities of the other lysosomal hydrolases did not vary significantly from those in the presence of 1 mU ADH ml⁻¹ (Table 1).

The effect of 1 mU ADH ml⁻¹ for 15 min in the presence of 50 p.p.m. antiserum specific for arginine vasopressin¹¹ was investigated. Control cells were incubated with antiserum alone. Activities of acid phosphatase and cathepsin in the incubation media of the hormone-treated groups were not significantly different from controls (Table 1). On the other hand, normal serum without hormone-specific antibody did not inhibit the

Table 1 Influence of ADH on lysosomal hydrolase activity in the extracellular medium of epithelial cells isolated from amphibian urinary bladder

Group	Incubation time (min)	Acid phosphatase activity (nmol per min per mg protein)	β-glucuronidase activity (nmol per min per mg protein)	Cathepsin B1 activity (nmol per min per mg protein)
Control	7.5	1.13 ± 0.03 (4)	3.04 ± 0.05 (5)	0.03 ± 0.01 (8)
ADH (1 mU ml ⁻¹)	7.5	1.87 ± 0.07 (4)*	3.77 ± 0.16 (5)†	0.08 ± 0.01 (8)*
Control	15	1.00 ± 0.10 (6)	3.19 ± 0.05 (5)	0.03 ± 0.01 (8)
ADH (1 mU ml ⁻¹)	15	1.37 ± 0.07 (5)†	3.67 ± 0.05 (3)*	0.07 ± 0.02 (8)‡
ADH (100 mU ml ⁻¹)	15	1.43 ± 0.07 (5)*	3.98 ± 0.10 (5)*	0.06 ± 0.00 (4)*
ADH antiserum (50 p.p.m.)	15	1.07 ± 0.07 (3)		0.03 ± 0.01 (3)
ADH antiserum and ADH (1 mU ml ⁻¹)	15	1.17 ± 0.03 (3)		0.03 ± 0.01 (3)

Activities of lysosomal enzymes are presented as mean ± s.e.m. with the number of determinations in parentheses. The significance of differences between hormone and control preparations was estimated by paired analysis with the Student's *t* test²⁴. In preliminary experiments, ADH was found to have no significant effect on enzyme activity *per se* in the assay conditions described in the text. In two additional experiments, 0.5 μg angiotensin II per ml, which is known to elicit a small hydro-osmotic effect in toad bladder²⁵, enhanced cathepsin activity to 110% of control cells incubated in the absence of the polypeptide for 15 min.

*Activity significantly different from control at *P* < 0.001.

†Activity significantly different from control at *P* < 0.01.

‡Activity significantly different from control at *P* < 0.05

Table 2 Early mucosal release of cathepsin B1 elicited by serosal addition of ADH to intact bullfrog urinary bladder

Group	Incubation time (min)	Cathepsin B1 activity (nmol per min per mg dry weight)	
		Serosal side	Mucosal side
Control	7.5	0.03 ± 0.02 (8)	0.01 ± 0.00 (8)
ADH (100 mU ml ⁻¹)	7.5	0.03 ± 0.02 (8)	0.03 ± 0.00 (8)*
Control	15	0.03 ± 0.00 (7)	0.01 ± 0.00 (7)
ADH (100 mU ml ⁻¹)	15	0.03 ± 0.01 (7)	0.02 ± 0.00 (7)*

Intact bullfrog urinary bladders were incubated as sacs with Ringer solution in isosmotic conditions and otherwise as described before²⁶. The activity of cathepsin B1 was determined on aliquots of particle-free supernatant fractions collected from the mucosal and serosal media bathing the bladder sac. Total enzyme activity per min in each experiment was then expressed relative to mg dry weight of the bladder sac. All activities are presented as mean ± s.e.m. with the number of determinations in parentheses.

*Value significantly different from control at $P < 0.001$.

ADH effect (not shown). In the presence of ADH antiserum, therefore, the hormone does not induce increments of lysosomal enzyme activities in the extracellular medium.

The early influence of ADH on the differential release of cathepsin into the media bathing the serosal and mucosal sides of intact bullfrog urinary bladder is shown in Table 2. Incubation with 100 mU ADH per ml serosal solution for 7.5 min elicited a significant increment in cathepsin activity in the mucosal, but not in the serosal, medium. This result is consistent with the observations on isolated cells presented in Table 1 and further implies that the hormone-enhanced release of lysosomal hydrolases occurs predominantly at the apical surface of amphibian bladder.

In view of the known lysosome-stabilising action of cortisol¹², the effect of 100 mU ADH ml⁻¹ was tested for 7.5 min in isolated cells that had been preincubated with 3.3×10^{-6} M cortisol for 40 min. Cortisol alone had no significant effect on the extracellular activities of acid phosphatase (not shown; $n = 3$) and cathepsin (Table 3) as compared with paired controls incubated with the steroid vehicle, ethanol, at a final concentration of 0.1%. With addition of ADH following cortisol, activities of phosphatase and cathepsin, respectively, rose $19 \pm 10\%$ ($P > 0.20$, $n = 3$) and 67% over the values of controls exposed only to cortisol. These data reveal that membrane stabilisation by cortisol results in marked reduction of the effect of ADH on lysosomal enzyme release. Other experiments presented in Table 3 further indicate that cortisol acts to antagonise ADH-induced changes in the transport and electrical properties of bullfrog urinary bladder. Cortisol alone had no statistically significant influence on water permeability, short-circuit current or membrane conductance as compared with paired control hemi-bladders during a 40-min incubation period. The normal increment in water transport and membrane conductance in response to ADH¹³ was markedly reduced in bladders incubated with cortisol before exposure to ADH. The steroid, however, did not alter the effect of ADH on short-circuit current. These

data are consistent with previous reports which suggest that ADH has independent actions on sodium and non-electrolyte transport¹³.

Experiments in progress in our laboratory indicate that partially purified cathepsin B can elicit increments in lectin-mediated cellular agglutinability (for example, increase in membrane fluidity) at pH 7.4 (ref. 4). The action of ADH on non-electrolyte permeability in amphibian bladder is likewise considered to occur subsequent to the enhancement of membrane fluidity^{4,13,14}. The observed redistribution of membrane-associated particles on the apical surface of frog urinary bladder epithelium after oxytocin treatment is in accord with this hypothesis¹⁵. Since limited proteolysis at the membrane surface is known to alter cellular agglutinability^{16,17} and membrane fluidity¹⁸ in other cell types, the membrane action of the lysosomal protease, cathepsin B, should be investigated further.

The release of lysosomal enzymes into extracellular media or body fluids is thought to occur by a process of exocytosis following lysosomal membrane labilisation¹⁹. There is precedent for hormone-enhanced liberation of soluble proteins²⁰ and of lysosomal hydrolases^{10,21,22} into the particle-free medium of their specific target cell preparations. The potential physiological significance of these enzymes in hormone action has been reviewed^{2,4,23}. These data, taken in context with the present observations, suggest that ADH-enhanced mucosal release of membrane-bound hydrolases may contribute to the increments in membrane permeability and fluidity which characterise the hormone response at the apical membrane of epithelial cells rich in intracellular secretion granules.

Isolation of the two major epithelial cell populations from bullfrog bladder by centrifugation procedures and subsequent treatment of viable cells with ADH indicates that the hormone elicits lysosomal hydrolase release exclusively from "granular" cells and not from mitochondria-rich cells (Pietras, Naujokaitis and Szego, unpublished).

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Table 3 Counteractive influence of cortisol on transport and electrical properties of bullfrog urinary bladder and extracellular release of lysosomal hydrolase from isolated epithelial cells following addition of ADH

Group	Extracellular cathepsin B1 activity (nmol per min per mg protein)	Water permeability coefficient $\times 10^7$ (cm s ⁻¹)*	Membrane conductance $\times 10^4$ (mho cm ⁻²)	Short-circuit current (μ A cm ⁻²)
Control (0.1% ethanol)	0.03 ± 0.00 (3)	1100 ± 100 (4)	3.8 ± 0.2 (4)	31 ± 3 (4)
ADH (100 mU ml ⁻¹)	0.07 ± 0.00 (3)†	6400 ± 100 (4)†	4.6 ± 0.2 (4)†	48 ± 1 (4)†
Cortisol (3.3×10^{-6} M)	0.03 ± 0.00 (3)	950 ± 50 (4)	3.6 ± 0.3 (4)	30 ± 2 (4)
Cortisol and ADH	0.05 ± 0.01 (3)‡	3100 ± 300 (4)†	3.7 ± 0.2 (4)	47 ± 1 (4)†

Radioactive tracer and electrical techniques were used to study the transport of water and sodium, respectively, across bullfrog bladders mounted as flat sheets between two Lucite chambers as described previously^{13,14}. Transport and electrical measurements were made during the 60-min period following addition of ADH, cortisol or both hormones (cortisol being present for 40 min in mucosal and serosal media before serosal addition of ADH). Extracellular cathepsin B1 activity was determined as described in the text at 7.5 min following treatment of isolated epithelial cells with ADH, cortisol or both hormones. Ethanol, 0.1% final concentration, was present in all media.

*Values corrected as described before¹³ for the presence of unstirred layers (that is 160 μ m) in the bullfrog urinary bladder (R.J.P., unpublished).

†Significantly different from control value of group immediately above in table at $P < 0.001$.

‡Significantly different from control value of group immediately above in table at $P < 0.01$.

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Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain

STUDIES on the pharmacology of the noradrenergic cyclic AMP-generating system in slices from the limbic forebrain of the rat¹ and on adaptive properties of this system in conditions of persistent changes in the availability of noradrenaline (NA) have revealed that the system may serve as a model for the central NA receptor in this area, and that its sensitivity to NA increases or decreases when the availability of NA at the receptor site decreases or increases respectively^{2,3}. Thus, hypersensitivity of the system has been achieved by treatment with reserpine², a drug known to

precipitate occasionally severe depressive reactions in man⁴, and the syndrome of which, when elicited in animals, is widely used as a model for depression^{5–7}. Conversely, the monoamine oxidase (MAO) inhibitors, pargyline and nialamide, caused a marked reduction in the reactivity of the cyclic AMP-generating system to NA after chronic administration³. To determine whether or not antidepressant drugs which do not elevate the level of monoamines in brain share the effect of MAO inhibitors on the noradrenergic cyclic AMP-generating system, we studied the effect of the tricyclic antidepressants, desipramine and iprindole, on the reactivity of the system to NA. Desipramine blocks the uptake of NA through the neuronal membrane^{8,9} whereas iprindole neither blocks the neuronal uptake of NA nor alters its metabolism or turnover^{10,11}, but is nevertheless a potent antidepressant^{12–14}. In addition, we have tested the effect of electroconvulsive treatment (ECT), as it is generally accepted to be one of the most effective treatments for severe depression¹⁵.

Male Sprague-Dawley rats (initial weight 180–220 g), housed four or five to a cage and kept in standard laboratory conditions (12-h light-dark cycle; food and water *ad libitum*) were used. The rats receiving antidepressant drugs were injected intraperitoneally with 10 mg kg⁻¹ d⁻¹ of desipramine or iprindole hydrochlorides for 4–8 weeks. Control rats received a corresponding volume of saline. Reserpine (Serpasil, CIBA-Geigy) was administered for 4 d at a dose of 2.5 mg kg⁻¹ d⁻¹. ECT was given by passing a current (100 mA; 300 ms) through ear clip electrodes. Controls had the electrodes attached, but no current was passed. Normal animals received ECT daily for 8 d, and the reserpine-treated animals received it for 4 d, approximately 2 h before each injection of reserpine.

The rats were decapitated at specified intervals after the last treatment, their limbic forebrain area (containing olfactory tubercles, septal and accumbens nuclei; anterior parts of the hippocampus and of the amygdaloid nuclei) was dissected as described previously², divided along the midline, chopped into blocks of approximately 0.5 mm³ and incubated at 37 °C following slightly modified² procedures of Kakiuchi and Rall¹⁶ and Palmer *et al.*¹⁷. After a 30-min preincubation period and change of the incubation medium (NaCl 118 mM; KCl 5 mM; CaCl₂ 2.5 mM; KH₂PO₄ 2 mM; MgSO₄ 2 mM; NaHCO₃ 24 mM; EDTA-Na 0.02 mM; glucose 10 mM; O₂-CO₂ 95:5; pH 7.4), the slices were incubated for an additional 14 min. At this time, slices from one half of the forebrain were exposed to a specified concentration of NA, whereas those from the corresponding

Table 1 Effect of antidepressant drugs and ECT on response to NA of cyclic AMP-generating system in limbic forebrain of rat

	Duration of treatment (d)	Time of death* (h)	NA concentration (μM)	Cyclic AMP response to NA† (pmol per mg protein ± s.e.m.)
Saline	28–56	24	5	23.9 ± 2.5 (12)
Desipramine (10 mg kg ⁻¹)	28–56	24	5	6.6 ± 2.0 (14)¶
Iprindole (10 mg kg ⁻¹)	28–56	24	5	7.2 ± 2.4 (16)¶
Handling	8	18	10	57.9 ± 1.9 (20)
ECT	8	18	10	40.3 ± 3.7 (20)‡
Handling	8	42	10	88.4 ± 6.2 (5)
ECT	8	42	10	44.4 ± 7.8 (5)¶
Handling	4	18	50	177.3 ± 14.1 (6)
Handling + reserpine (2.5 mg kg ⁻¹)	4	18	50	268.2 ± 17.6 (7)§
ECT + reserpine (2.5 mg kg ⁻¹)	4	18	50	208.8 ± 17.2 (7)¶

*Time after last injection of drug, ECT or handling.

†Difference of levels of cyclic AMP between preparation exposed to NA and non-exposed preparation from corresponding half of the limbic forebrain. Numbers in parentheses denote number of pairs. Basal levels of cyclic AMP were not changed by the antidepressants or ECT (17.8 ± 2.6 pmol per mg protein in saline-treated and 35.1 ± 1.4 pmol per mg protein in handled group).

‡P < 0.05.

§P < 0.01.

¶P < 0.001 (difference between control and experimental; drug and/or ECT group).

||Not significantly different from control (handled group), but different (P < 0.05) from handled, reserpine-treated group.

half served for the determination of the basal level of cyclic AMP. The reaction was terminated 10 min later by homogenisation of the tissue slices in 3.5 ml 0.3 N HClO₄; a 0.5-ml aliquot of the homogenate was used for the determination of the protein concentration by the method of Lowry¹⁸. Cyclic AMP was isolated by ion-exchange chromatography and assayed by the method of Gilman¹⁹. All values were corrected for recoveries and cyclic AMP was expressed in pmol per mg protein. Student's two-tailed *t* test was used for the statistical evaluation of the data.

The effect of chronic treatment with tricyclic antidepressants and of ECT is shown in Table 1. Prolonged treatment with antidepressant drugs reduced the responses of the cyclic AMP-generating system by approximately 70%. A much shorter treatment with ECT reduced the response by 30% in rats killed 18 h after the last shock, and, interestingly, by 50% in rats killed a day later. Reserpine treatment (2.5 mg kg⁻¹ daily for 4 d) enhanced the response to NA by approximately 50%, confirming earlier results². Concomitant ECT practically abolished the increased cyclic AMP response to NA (Table 1). The response to NA has not been significantly changed in preparations from rats receiving tricyclic antidepressants for a period of 2 weeks or less. Our results complement data obtained with MAO inhibitors, which depressed the cyclic AMP response to NA by 50–60% after prolonged but not short term treatment³ and reveal a common mode of action at post-synaptic noradrenergic receptor sites for antidepressants regardless of their action at presynaptic sites, and for ECT. The decreased reactivity to NA after ECT may provide the biochemical basis for the antagonism by ECT of stimulant effects induced by combined treatment with desipramine and Ro4-1284 (ref. 20) or amphetamine²¹, and for the potentiation by ECT of the reserpine syndrome and α -methyltyrosine-induced catalepsy²¹.

Our data emphasise the dissociation of acute biochemical effects of antidepressant drugs from their therapeutic action, which occurs only after chronic treatment for weeks. The demonstrated decrease in noradrenergic receptor function as a consequence of various antidepressant treatments—pharmacotherapy and ECT—should focus future neurochemical research on the elucidation of molecular mechanisms of altered aminergic receptor function and provide a new theoretical framework for studies on both pathogenesis and therapy of affective disorders.

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Tubulin in postsynaptic junctional lattice

TUBULIN, the primary protein of microtubules^{1,2}, has been identified as a major soluble protein in isolated synaptosomes^{3,4}. Indirect evidence for the presence of tubulin in the synaptosomal plasma membranes themselves has been accumulating for some time. Colchicine-binding activity, used as an assay for the presence of tubulin^{1,2}, has been found in synaptosomal plasma membranes^{5–7}. These membranes, prepared by several different techniques, all show a prominent protein band of apparent molecular weight 52,000–53,000 when solubilised and electrophoresed in sodium dodecyl sulphate–polyacrylamide gel systems^{8–12}; such gel techniques applied to well characterised microtubule preparations yield molecular weights for tubulin which have been reported to range from 52,000 to 56,000 (refs 1 and 2), the precise value depending on the particular

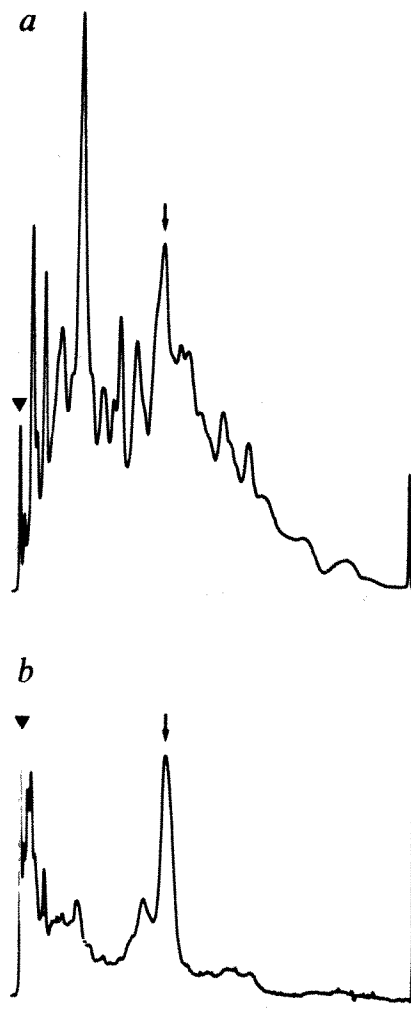


Fig. 1 Densitometer scans of the proteins of synaptosomal plasma membranes (a) and isolated postsynaptic junctional lattices (b), stained with Coomassie brilliant blue after electrophoresis in 7.5% polyacrylamide gels with a continuous buffer system of 0.1% (w/v) sodium dodecyl sulphate, 0.1 M Tris-HCl, pH 7.4. The sample buffer contained dodecyl sulphate–protein 2:1 (w:w) and 0.2% 2-mercaptoethanol in 5mM Tris-HCl, pH 7.4. Triangles indicate the tops of the gels, and the arrows indicate the bands with an apparent molecular weight of 50,300.

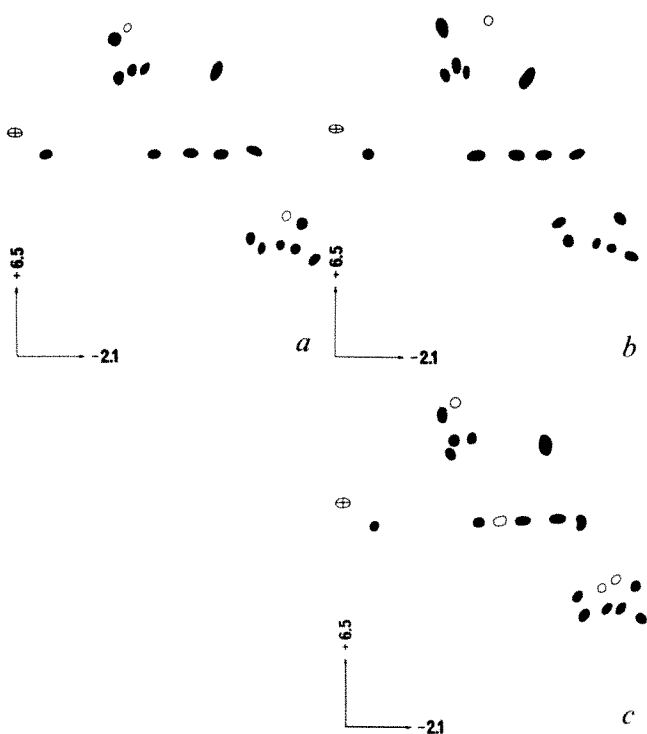


Fig. 2 Tracings of autoradiographs of the tryptic maps obtained from proteins labelled with ^{125}I after electrophoresis in sodium dodecylsulphate-polyacrylamide gels¹⁸. Crossed circles mark the origins, solid circles the peptides common to all three maps and open circles the remainder. The starting proteins were: *a*, the 50,300-dalton protein from synaptosomal plasma membranes; *b*, the 50,300-dalton protein from isolated postsynaptic junctional lattices; *c*, electrophoretically pure tubulin.

electrophoretic system used. Sequential solubilisation of synaptosomal plasma membranes with Triton X-1000 and N-lauroyl sarcosinate yields isolated postsynaptic densities¹³ whose major protein has a molecular weight of 53,000 (ref. 14), and similarly digestion of the synaptosomal lipid unit membrane by sodium deoxycholate results in isolated postsynaptic junctional lattices¹⁵ with a major protein of approximate molecular weight 53,000 (ref. 12). We report here the identification of tubulin in the postsynaptic junctional lattices of rat forebrain both by two-dimensional electrophoresis of ^{125}I -labelled tryptic peptides and by electron microscope immunohistochemistry.

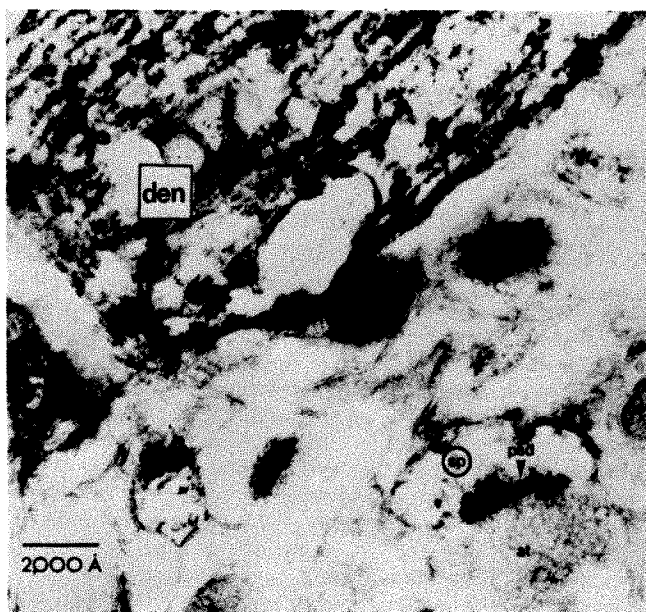
Synaptosomal plasma membranes were prepared from rat forebrain by the method of Jones and Matus¹⁶, with the slight modifications that all sucrose solutions and the lysis buffer contained 50 μM calcium chloride and the sucrose solutions were also buffered with 5 mM HEPES, pH 7.4. Postsynaptic junctional lattices were isolated by incubating the membranes at a final protein concentration of 1.7 mg ml⁻¹ in 10% (w/v) sodium deoxycholate, 1 mM phenylmethylsulfonylfluoride, and 50 μM calcium chloride for 2 h at 37 °C, followed by dilution with a two-thirds volume of distilled, deionised water and pelleting at 100,000g for 1 h at 4 °C (see ref. 12). Of the membrane protein, 98% is solubilised by this procedure. Figure 1 shows densitometer scans of the proteins of the starting synaptosomal plasma membranes and the isolated postsynaptic lattices, stained with Coomassie brilliant blue after electrophoresis in sodium dodecyl sulphate-polyacrylamide gels. The band in each gel indicated by the arrow has an apparent molecular weight of 50,300 and comigrates with added rat brain tubulin prepared by the repeated polymerisation method of Shelanski *et al.*¹⁷. These bands were excised from the gels, particular care being taken to omit the shoulder (molecular weight 54,300) on the membrane

peak. The tubulin band from a similar gel of electrophoretically pure tubulin, itself prepared by elution from polyacrylamide gels of once repolymerised tubulin, was also excised. The proteins from these slices were eluted, concentrated, iodinated by a chloramine T reaction, performic acid oxidised and then digested with trypsin and the resultant peptides purified, using the methods of Bray and Brownlee¹⁸. Samples of these peptides were electrophoresed at 3,000 V in 2 dimensions on Whatman 3 MM paper, first for 75 min in pH 6.5 buffer (10% pyridine, 0.3% acetic acid) and then for 75 min in pH 2.1 buffer (8% acetic acid, 2% formic acid). The electrophoretograms were exposed to X-ray film for 42–168 h. Figure 2 shows the autoradiographic maps of the tyrosine-containing tryptic peptides of the 50,300-dalton protein from synaptosomal plasma membranes, the 50,300-dalton protein from postsynaptic junctional lattices, and tubulin. The number of spots in the tubulin map (20) agrees well with the expected number of tubulin tyrosine peptides (18–19) (ref. 19) and the number of iodinated peptides previously reported (18–20) (ref. 18). The three tryptic fingerprints are almost identical, and thus the three proteins from which they were derived are strikingly homologous.

Approximately 300 μg of tubulin which had been excised from polyacrylamide gels of once-repolymerised rat brain tubules, eluted, and concentrated, was intramuscularly injected into a rabbit in 50% Freund's complete adjuvant and comparable booster shots administered at 10-d intervals. This technique has been used to produce monospecific antibodies to actin²⁰ and myosin²¹. Figure 3 is an electron micrograph immunohistochemically illustrating the cellular localisation of the rat brain tubulin antigen in rat cerebral cortex²². The peroxidase reaction product is clearly visible on dendritic microtubules and is also present at the postsynaptic lattice of the visible axodendritic synaptic junction. In all the material we have examined, incubation of brain tissue with anti-tubulin serum in the immunoperoxidase procedure produced staining only in association with microtubules and the postsynaptic junctional lattices²³.

We have thus established that the major postsynaptic

Fig. 3 Electron micrograph of a rat cerebral cortex slice which was incubated with rabbit anti-tubulin serum, washed with phosphate buffered saline (PBS), incubated with peroxidase-labelled sheep anti-rabbit gamma globulin serum, washed again with phosphate-buffered saline, and the immunohistochemical staining developed by 30 min exposure to a solution of 5 mg 3,3'-diaminobenzidine in 10 ml of PBS, pH 7.2, to which had been added 10 μl of hydrogen peroxide.



junctional lattice protein and tubulin share a common antigen and are so closely similar in primary structure that their tryptic digests are virtually identical. These results confirm the earlier tentative identification of tubulin as the major component of the postsynaptic density, where it probably has an important structural role in providing a matrix for more specialised proteins of functional importance in synaptic transmission^{12,14}. Some form of functional role for the tubulin itself cannot, however, be discounted at present. It has been proposed that "linkage elements" associated with the cell membrane and pharmacologically similar to tubulin are involved in the selective cellular control of particular membrane components²⁴. Although such elements may be present throughout the synaptosomal plasma membrane, more of these "tubulin-like" molecules would be expected in specialised areas, especially ordered arrays such as are present at the synaptic junction¹⁵. The observed distribution of tubulin in the synaptosomal plasma membrane and postsynaptic junctional lattice precisely matches the hypothesis that tubulin functions as a membrane-associated molecular linkage element.

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Post-transcription control of isocitrate lyase induction in the eukaryotic alga *Chlorella fusca*

WE have shown previously¹ that poly(A)-containing RNA can be isolated from the green alga *Chlorella fusca* 8p (ref. 2) and that this RNA has messenger-like properties, as it is polydisperse and stimulates polypeptide synthesis *in vitro* in a wheat embryo system. We show here that the *Chlorella* protein isocitrate lyase has been identified as a product of *in vitro* translation of poly(A)-containing RNA, so confirming messenger function in this fraction. We have used the *in vitro* translation system as a means of assaying for the presence of isocitrate lyase message sequences in poly(A)-containing RNA isolated from *Chlorella* cultured under different conditions, and we report here that slow-growing non-induced cells may contain high levels of mRNA for this enzyme although the enzyme is not synthesised.

Isocitrate lyase is an adaptive enzyme^{3,4}, and its synthesis

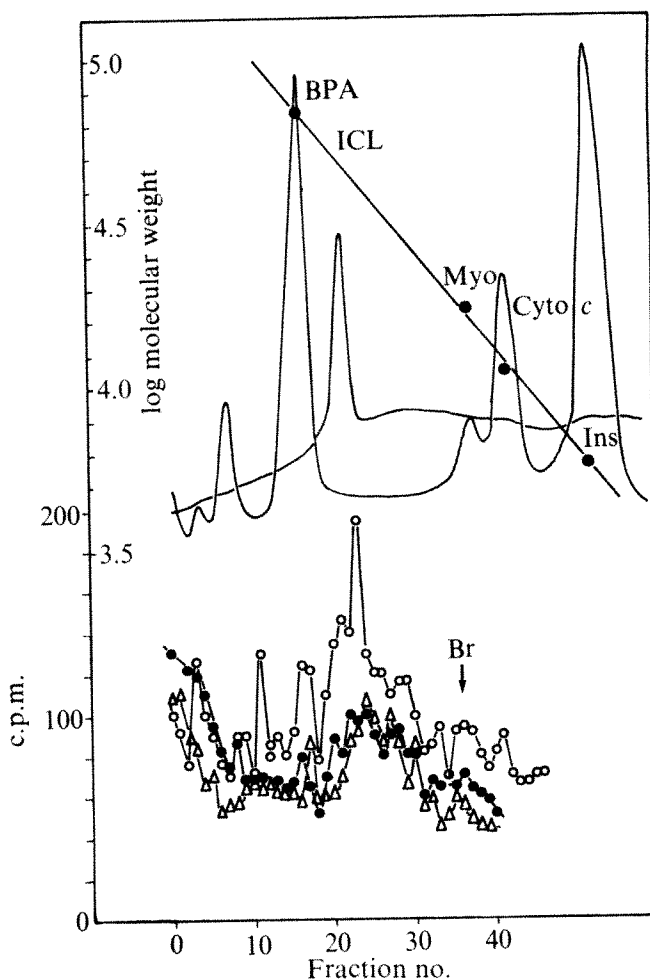


Fig. 1 SDS gel electrophoresis of immunoprecipitated protein produced by *in vitro* protein synthesis, in a wheat embryo system. Total *Chlorella* RNA was separated on a cellulose column into an unadsorbed fraction and an adsorbed fraction (described fully in ref. 1). The adsorbed fraction was poly(A)-containing RNA of which 46 µg were added to one cell-free protein synthesis assay (○). The unadsorbed fraction from the column was mostly rRNA of which 50 µg were added to a second cell-free protein synthesis assay (△). A control assay where no RNA was added was also performed (●). *Chlorella* RNA was isolated as described previously¹, from a culture containing 2.5×10^7 cells ml⁻¹ which had been incubated in the dark with 0.1% sodium acetate for 90 min (acetate-adapting *Chlorella*). The cell-free protein synthesis mixtures were prepared by the method of Roberts and Paterson¹⁰ as described previously¹, but scaled up to a total volume of 400 µl, and were incubated at 30 °C for 2 h. The radioactive amino acid included was ³⁵S-methionine (10 µCi, 200 Ci mmol⁻¹). After incubation all assays were treated as follows: 5 µg ovalbumin and 50 µl anti-ovalbumin serum were added and precipitates formed after 2 h at room temperature were removed by centrifugation (preprecipitation, see text). Supernatants were mixed with carrier antigen (5 µl pure isocitrate lyase, 0.75 mg ml⁻¹) and 25 µl of specific anti-isocitrate lyase serum. After 16 h at 4 °C the immune precipitate was recovered by centrifugation, washed three times with 1 ml of 5 mM Tris-acetate, pH 7.5, dissociated with 20 µl of 0.2 M NaOH for 15 min at room temperature and neutralised by addition of 20 µl of 0.2 M HCl and 60 µl of 8 M urea. The resulting mixtures were made 1% in sodium dodecyl sulphate (SDS) and 0.01 M in 2-mercaptoethanol and incubated at 100 °C for 2 min before electrophoresis in 7.5% polyacrylamide gels¹¹. After electrophoresis the gels were sliced into 1-mm segments and the radioactivity in each slice was determined as previously described¹. Br, Bromophenol blue marker. The upper part of the figure shows absorbance at 650 nm of two gels run under identical conditions and loaded with pure isocitrate lyase (ICL) or insulin (Ins), cytochrome c (Cyto c), myoglobin (Myo) and bovine plasma albumin (BPA). Both gels were stained with Coomassie brilliant blue.

is induced in *Chlorella* when acetate is added to darkened cultures. There is temporal restriction of isocitrate lyase synthesis in synchronous *Chlorella* cells³ and its synthesis is prevented when photosynthesis is possible⁶ or when glucose is present in the medium^{3,4}, indicating a catabolite repression control mechanism in addition to the inducing effect of acetate. When fully induced, isocitrate lyase is an unusually abundant enzyme constituting 7% of total soluble protein⁷. This suggested that isocitrate lyase mRNA might be a relatively abundant RNA species.

Preliminary experiments showed that rabbit antiserum which was raised against isocitrate lyase purified to homogeneity⁸, precipitated less than 0.05% of the radioactivity in an extract from an autotrophic *Chlorella* culture grown in the presence of ³⁵S-sulphate⁹, when the antiserum was added in the presence of sufficient unlabelled pure isocitrate lyase to enable immune precipitation. When unlabelled pure isocitrate lyase was added to wheat embryo cell-free protein synthesis assays and precipitated with specific antiserum, the immune precipitate was contaminated with proteins resulting from nonspecific adsorptions. In the experiment described under Fig. 1 this nonspecific effect was reduced by a preprecipitation step, where products of cell-free protein synthesis assays were mixed with ovalbumin which was precipitated with a specific anti-ovalbumin serum.

Figure 1 shows the results of electrophoretic analysis of the proteins precipitated from cell-free protein synthesis assays by anti-isocitrate lyase serum. The addition to the cell-free system of poly(A)-containing RNA isolated from *Chlorella* cultures in the process of isocitrate lyase synthesis, resulted in synthesis of a protein *in vitro*, which was absent from assays which had received either no added RNA, or *Chlorella* rRNA. The peak of radioactive protein had the same electrophoretic mobility as pure isocitrate lyase, which is also shown in Fig. 1. Isocitrate lyase is a tetrameric protein and under the conditions used to dissolve the immune precipitate, it was completely dissociated into subunits of molecular weight ~ 47,000.

To minimise the problem of nonspecific adsorption of proteins to the antibody, the anti-isocitrate lyase serum was immobilised by cyanogen bromide linkage to Sepharose beads (anti-ICL-Sepharose). When pure isocitrate lyase was adsorbed by a column of anti-ICL-Sepharose, only a small proportion of the enzyme was displaced by 0.01 M NaOH whereas the remainder was displaced quantitatively with 0.1 M NaOH (Fig. 2). Figure 2 also shows the elution of radioactive protein from an anti-ICL-Sepharose column which had been loaded with the products of a cell-free protein synthesis assay, primed with poly(A)-containing RNA isolated from *Chlorella* in the process of isocitrate lyase synthesis. Gel electrophoresis of protein eluted by 0.1 M NaOH is shown in Fig. 3, where it can be seen that a profile of peaks closely resembling those obtained with pure isocitrate lyase was obtained. Preparation of samples for electrophoresis as performed in this experiment did not completely dissociate isocitrate lyase and a distribution of monomers and multimers was obtained. It is evident that protein adsorbed to the anti-ICL-Sepharose column and

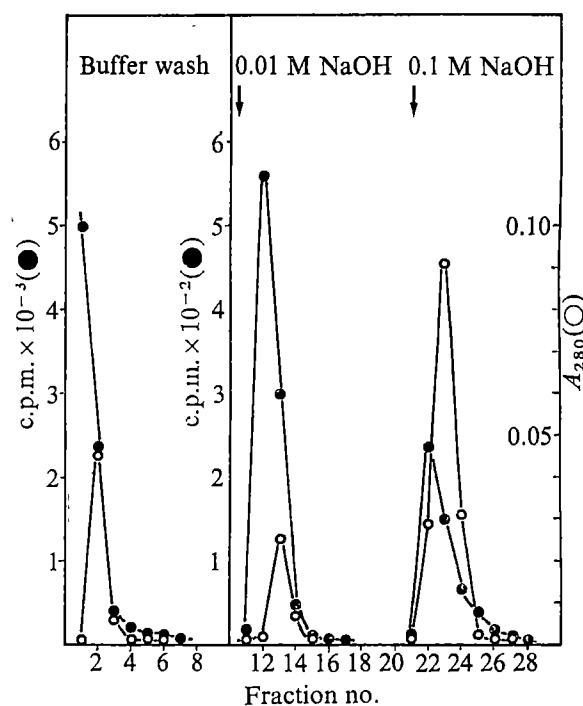


Fig. 2 Elution of products of *in vitro* protein synthesis from a column of anti-isocitrate lyase serum covalently linked to sepharose (anti-ICL-Sepharose). The data are for products of *in vitro* translation of poly(A)-containing RNA from acetate-adapting *Chlorella*, in a 200 μ l cell-free mixture incubated at 30 °C for 3.5 h, but otherwise as described under Fig. 1, (●). Pure isocitrate lyase was also adsorbed to a column and eluted (○). To prepare anti-ICL-Sepharose, 2 ml of anti-isocitrate lyase serum (15 mg ml⁻¹ protein) were linked to 1 g of cyanogen bromide-activated Sepharose 4B following the manufacturer's instructions (Pharmacia). Anti-ICL-Sepharose, suspended in 0.5 M NaCl, 0.005 M sodium phosphate, pH 7.0 was packed in 1-ml columns and washed with 5 ml of 0.005 M sodium phosphate, pH 7.0 (phosphate buffer) before use. Products of cell-free protein synthesis assays or pure isocitrate lyase (40 μ g) were applied to the column and allowed to adsorb for 1 h at room temperature, before washing through with 20 ml of phosphate buffer (buffer wash). Elution with 2 ml of 0.01 M NaOH was followed by a further 20 ml of phosphate buffer (0.01 M NaOH eluate). Lastly 2 ml of 0.1 M NaOH were applied and this also was followed with 20 ml of phosphate buffer (0.1 M NaOH eluate). Hot trichloroacetic acid-insoluble radioactivity in each fraction was counted as previously described¹. Elution of pure isocitrate lyase was followed by measurement of absorbance at 280 nm.

which was only displaced by the higher NaOH concentration with largely isocitrate lyase. This, in turn, demonstrated the presence of message sequences for isocitrate lyase in poly(A)-containing RNA from *Chlorella*.

In the conditions of batch culture which we used⁸, autotrophic *Chlorella* cultures grew exponentially until cell number density reached about 10⁷ cells ml⁻¹, then growth rate declined as they became more and more light limited, finally ceasing at a density of about 5 × 10⁷ cells ml⁻¹. At no stage of growth does isocitrate lyase synthesis occur

Table 1 Fractionation of *in vitro* translation products of *Chlorella* poly(A)-containing RNA on anti-ICL-Sepharose columns

Source of poly(A)-containing RNA	Buffer wash	Hot TCA-insoluble radioactivity (c.p.m.)		% of total c.p.m. in 0.1 M NaOH fraction
		0.01 M NaOH eluate	0.1 M NaOH eluate	
(1) Acetate-adapting cells	103,600	25,000	13,500	9.4
(2) Control (minus RNA)	50,000	530	1,900	3.6
Exponential autotrophic cells	50,900	5,400	1,600	3.0
Light-limited autotrophic cells	44,100	2,800	5,800	12.3

Data assembled from Fig. 2 (1) and Fig. 4 (2). Pooled fractions were sampled and hot trichloroacetic acid-insoluble radioactivity measured. For (1) cell-free translation mixtures were incubated for 3.5 h at 30 °C. For (2) cell-free translation mixtures were incubated for 2 h at 30 °C.

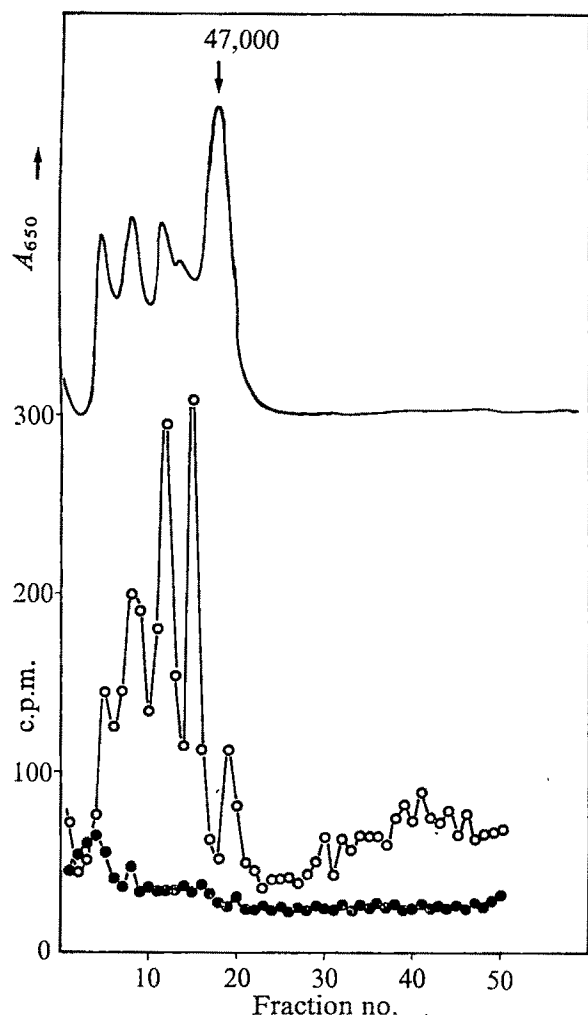


Fig. 3 SDS disc gel electrophoresis of *in vitro* translation products eluted with 0.1 M NaOH from anti-ICL-Sepharose columns (see Fig. 2). *In vitro* translation of poly(A)-containing RNA from acetate-adapting *Chlorella* is described in Fig. 1 and fractionation of the translation products on anti-ICL-Sepharose is shown in Fig. 2. Protein in the 0.1 M NaOH eluate was subjected to electrophoresis (○). Products of a control *in vitro* translation assay (minus RNA) were also fractionated (Fig. 4) and protein in the 0.1 M NaOH eluate subjected to electrophoresis (●). Both samples were prepared for electrophoresis as follows: pooled column fractions were made 5% in trichloroacetic acid; precipitated protein was recovered by centrifugation, washed twice with 5 ml of ethanol, resuspended in gel buffer¹² containing 1% SDS and 0.01 M 2-mercaptoethanol and heated to 100 °C for 2 min. Electrophoresis was carried out in 15% SDS gels¹² after which they were cut into 1-mm slices which were incubated overnight at 37 °C in a toluene-based scintillation mixture including 5% Protosol (New England Nuclear) and counted for radioactivity. The upper part of the figure shows absorbance at 650 nm of a gel which had been run with 20 µg of pure isocitrate lyase and stained with Coomassie brilliant blue.

unless acetate is the sole available carbon and energy source.

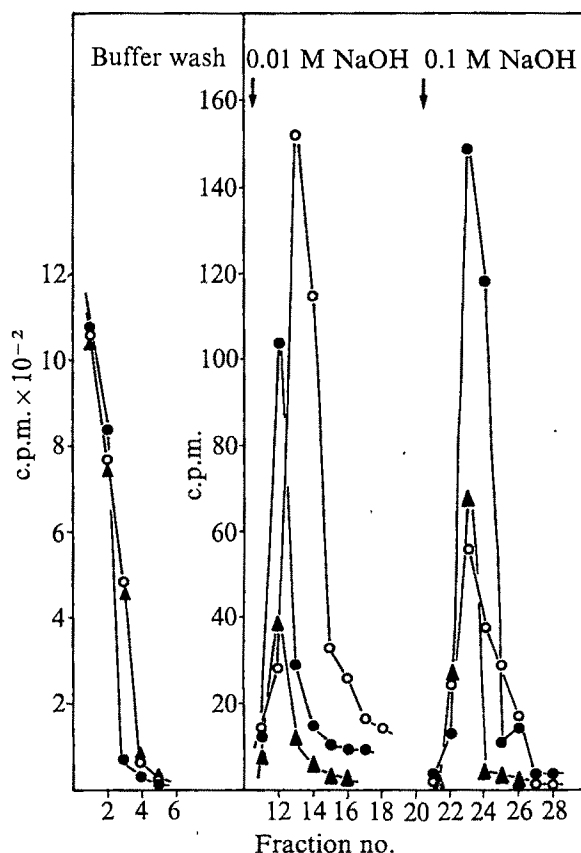
Poly(A)-containing RNA was isolated both from exponentially growing autotrophic cultures and from light-limited cultures. Analysis of products of *in vitro* protein synthesis directed by these RNA samples, using anti-ICL-Sepharose columns, is shown in Fig. 4. When poly(A)-containing RNA from exponentially growing cells was tested, the amount of radioactive protein in the 0.1 M NaOH eluate from the column was the same as the control, in which the cell-free protein synthesis assay was performed without added RNA (see also Table 1). In contrast, addition to the cell-free system of poly(A)-containing RNA isolated from light-limited autotrophic cells resulted in isocitrate lyase synthesis as demonstrated by the larger amount of

radioactive protein in the 0.1 M NaOH eluate (Fig. 4 and Table 1). In a separate experiment (results not shown) this protein was found to have the same electrophoretic mobility as pure isocitrate lyase (analogous to Fig. 3).

The absence of detectable isocitrate lyase mRNA in exponentially-growing, photosynthesising cells and its presence in cells adaptively synthesising isocitrate lyase, provides evidence for the operation of a transcriptional control, but it is also firmly demonstrated that a post-transcriptional control can operate, since messenger accumulates without being translated, as low light limits the growth rate.

When *Chlorella* cells use acetate as their sole carbon source, the activity of the glyoxylate cycle is necessary for the synthesis of intermediary metabolites which are normally more readily produced by photosynthesis or the metabolism of glucose¹³. The synthesis of isocitrate lyase correlates with the detection of glyoxylate cycle activity in this strain of *Chlorella*³ and the present study demonstrates that the adaptive synthesis of the enzyme in rapidly growing cells follows the accumulation of mRNA from previously undetectable levels. This evidence provides further support for the conclusion that synthesis of the enzyme in rapidly growing cells is initiated by transcription, which was previously drawn tentatively from the effect of inhibiting functional RNA synthesis⁴. There is therefore a parallel with the control of gene expression in prokaryotic cells¹⁴⁻¹⁶.

Fig. 4 Elution from anti-ICL-Sepharose of *in vitro* translation products of poly(A)-containing RNA extracted from autotrophically grown *Chlorella*. Poly(A)-containing RNA was isolated¹ from an autotrophic *Chlorella* culture collected during exponential growth (cell number density, 6.5×10^6 cells ml⁻¹), (○) and from a culture which had become light-limited (cell number density, 1.44×10^7 cells ml⁻¹) (●). These poly(A)-containing RNA samples were translated in the wheat embryo cell-free system in a final volume of 200 µl, but otherwise as described in Fig. 1. Translation products were fractionated on anti-ICL-Sepharose columns as described in Fig. 2. Products of a control cell-free assay to which *Chlorella* RNA had not been added, were also fractionated (▲). Hot trichloroacetic acid-insoluble radioactivity was measured.



The direct estimation of isocitrate lyase mRNA, however, provides evidence for at least one additional control point acting at the post-transcriptional level, which is more specific in its requirements for release of enzyme synthesis. In light-limited cells the supply of intermediary metabolites is sub-optimal and the accumulation of isocitrate lyase mRNA is initiated, but this is not sufficient to cause synthesis of the enzyme. Therefore the restriction of isocitrate lyase synthesis to conditions when acetate is present to act as substrate for the glyoxylate cycle and with no alternative carbon source available, is achieved by the operation of post-transcriptional control. This control may be lacking in the thermophilic strain of *Chlorella*, in which darkening without addition of acetate is sufficient to promote maximum synthesis of isocitrate lyase¹⁷.

It is possible that the mRNA which is accumulated, but not translated, in light-limited cells is held at a stage requiring further processing, such as specific cleavage from a large nuclear precursor (HnRNA), or export from the nucleus. This interpretation supposes that the wheat embryo *in vitro* protein-synthesising system can translate unprocessed messenger, as can *Xenopus* oocytes¹⁸. There is evidence, however, that post-transcriptional control is exerted when mRNA is fully processed and is already supporting isocitrate lyase synthesis. Glucose, which makes the glyoxylate cycle redundant, causes an immediate cessation of enzyme synthesis^{4,6}, in contrast to the gradual cessation of synthesis which follows the use of an inhibitor to prevent further transcription⁴. Many metabolite levels in *Chlorella* are altered by changes in carbon source^{19,20} and these metabolites could operate post-transcriptional control by modifying the binding of a regulator protein to mRNA^{21,22}, or by modifying the nascent protein to produce either abortive folding²³ or enhanced sensitivity to protease attack²⁴.

Whatever the precise mechanism of post-transcriptional control for isocitrate lyase synthesis, the existence of this control in eukaryotic algae, taken together with evidence for the operation of similar mechanisms in mammalian cells²⁵, suggests that post-transcriptional controls may be common in eukaryotes.

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Is Fanconi's anaemia defective in a process essential to the repair of DNA cross links?

FANCONI'S anaemia (FA) is a rare autosomal recessive disease of man, characterised by a progressive hypoplastic pancytopenia associated with diverse congenital anomalies^{1,2}, spontaneous chromosome breakage³ and predisposition to leukaemia and other cancers^{4,5}. Lymphocytes from FA patients were found to be excessively susceptible to chromosome breakage by di- or polyfunctional alkylating agents^{6,7}, and this was interpreted as a possible indication of defective DNA repair of the FA cells⁷. Seeking evidence for this, I have tested lymphocytes from FA patients for their chromosome response to mono- and difunctional mitomycins in relation to the cell cycle phase at the time of treatment, and have found that FA cells have a specific defect in the repair of pre-aberration lesions induced by difunctional mitomycin; the lesions are possibly DNA cross links of the interstrand type. The rationale of this experiment was the knowledge that if damage to DNA is left unrepaired it can be linked causally to the formation of chromosome aberrations by a process that resides in semiconservative DNA replication⁸⁻¹². Since the repair of DNA damage is a rate-limiting process, the treatment of repair-proficient cells in early G₁ would leave more time for repair before the damage is fixed into chromosome aberrations during the S phase, and consequently would result in fewer chromosome aberrations than treatment during the transition from G₁ to S. In the repair-deficient cells, however, DNA damage induced by treatment in any position of G₁ would be linked maximally to the formation of chromosome aberrations.

Heparinised venous blood was obtained from two male patients with FA aged 6 and 7 yr (identified as FA7 and FA9) and a normal healthy person as a control. The procedure has been described before¹⁰. Briefly, a series of whole-blood microcultures was set up using basal medium NCTC-109 supplemented with 20% foetal calf serum and 3% phytohaemagglutinin, and fixed for chromosome preparation after 70-74 h of incubation. At various times before fixation, cells were treated for 30 min with difunctionally reacting mitomycin C (MMC) or its monofunctional derivative, decarbamoyl mitomycin C (DCMMC, a gift from Dr K. Nakano, Kyowa Hakko Kogyo Co.) in conjunction with ³H-thymidine, and incubation was continued for a corresponding recovery time in culture medium free of test compounds. The preparations were processed for autoradiography and analysed for their metaphase labelling pattern and the presence of chromosome aberrations in labelled and unlabelled mitoses.

The changes in metaphase labelling index are shown in Fig. 1. Even in the absence of mitomycins, FA cells passed more slowly than normal through S and G₂. Moreover, in contrast to normal cells, most FA cells collected at 70-74 h were still in their first mitosis, as indicated by a comparatively small amount of the second maximum metaphase labelling index. Treatment with mitomycins reduced the rate of progression through the cell cycle, and the effect was more marked when the FA cells were treated with MMC. Figure 2 shows that ³H-thymidine alone had little effect on chromosomes of normal or FA cells. Although DCMMC was less efficient than MMC, both induced significant chromosome aberrations of the chromatid type. The

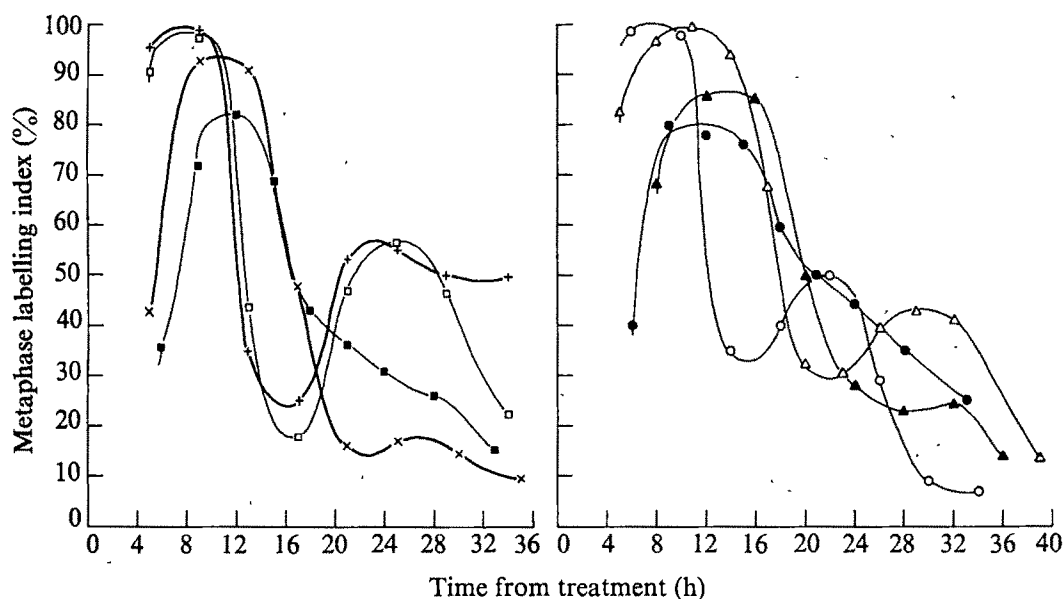


Fig. 1 Changes in the proportion of labelled mitoses as a function of time from treatment. At various times before fixation, cells were treated for 30 min at 37 °C with DCMMC or MMC which was made up in medium TC-199 containing ^3H -thymidine ($1 \mu\text{Ci ml}^{-1}$) or with ^3H -thymidine alone, washed and re-incubated for the indicated recovery time in the medium NCTC-109 supplemented with 20% foetal calf serum and $5 \times 10^{-5} \text{ M}$ cold thymidine. All cultures were fixed at any time between 70 and 74 h of total incubation time with a 2-h prefixation treatment with colchicine. Chromosome preparations were made according to the standard air-drying technique using hypotonic treatment in

0.9% sodium citrate before fixation in methanol-acetic acid (3:1). Preparations were processed for autoradiography using Kodak NTB3 emulsion. Metaphase labelling index was determined on 250-400 mitoses. +, Normal cells treated with ^3H -thymidine alone; \times , FA9 cells treated with ^3H -thymidine alone; \square , normal cells treated with DCMMC ($20 \mu\text{g ml}^{-1}$); \blacksquare , FA7 cells treated with DCMMC ($50 \mu\text{g ml}^{-1}$); \circ , normal cells treated with MMC ($0.5 \mu\text{g ml}^{-1}$); \triangle , normal cells treated with MMC ($1.5 \mu\text{g ml}^{-1}$); \bullet , FA7 cells treated with MMC ($0.1 \mu\text{g ml}^{-1}$); \blacktriangle , FA9 cells treated with MMC ($0.1 \mu\text{g ml}^{-1}$).

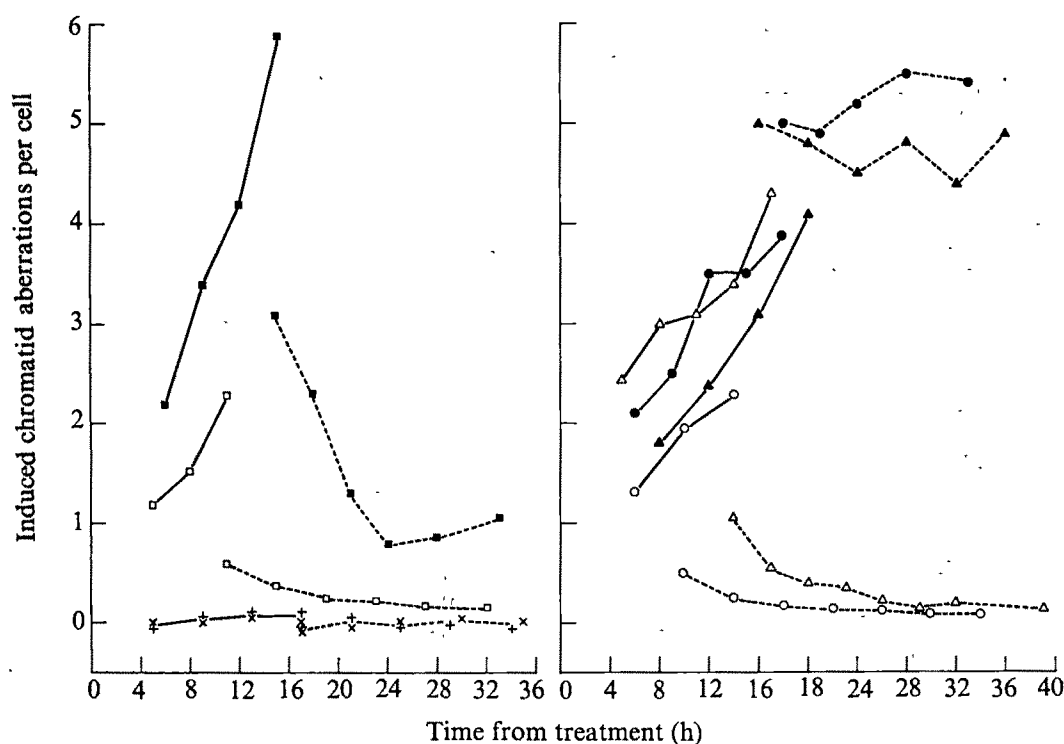


Fig. 2 Changes in the frequency of chromatid aberrations in labelled (solid lines) and unlabelled (dashed lines) mitoses as a function of time after treatment. After recording of the labelling-pattern and location of mitoses, the silver grains were removed and chromatid aberrations were scored in the preselected mitoses (100 for each point). Chromatid aberrations scored are gaps, breaks and exchanges occurring at the chromatid level. The scoring does not include the cells in S phase at the time of

treatment and recovered at their second mitosis. The frequencies of induced aberrations are expressed as the net amount of induced aberrations, that is, observed frequencies minus spontaneous levels, which were 0.07, 2.00 and 2.14 per normal, FA7 and FA9 cell, respectively, as determined in their 72-h cultures, which were kept untreated during incubation. The symbols used are the same as in Fig. 1

patterns of the chromosome response of normal cells to DCMC and MMC and that of FA cells to DCMC were as predicted for repair-proficient cells. With respect to formation of chromosome aberrations, the S phase, more particularly its very beginning, was the most sensitive stage. The chromosome aberrations were less frequent in unlabelled mitoses that were in the pre-DNA-synthesis stage at the time of treatment, and their frequencies varied with the recovery time, being maximum in late G₁, decreasing with increasing recovery time. When tested with MMC, however, FA cells were most sensitive at G₁ and, moreover, yielded a constant rate of aberrations when treated in various parts of G₁, and a decreasing yield as the S phase progressed. This pattern of chromosome response to MMC is comparable with that of xeroderma pigmentosum cells to 4-nitroquinoline-1-oxide¹⁰. These cells have an impaired capacity to repair DNA alterations induced by ultraviolet light and some chemicals, including 4-nitroquinoline-1-oxide^{13,14}. These results indicate that in the FA cells pre-aberration lesions induced by DCMC can be repaired, but those induced by MMC at any time before DNA synthesis are not repaired and link maximally to the chromosome aberrations formed during the subsequent S phase. Since the FA cells are also abnormally susceptible to chromosome breakage by the psoralen-plus-light reaction⁷, which is highly specific to native DNA and induced interstrand cross links¹⁵, it is probable that the MMC-induced lesions that are irreparable in the FA cells are DNA cross links of the interstrand type.

In bacteria, repair of cross links is controlled by *uvr* and *recA* genes, and it has been suggested to be mediated by a sequential process involving excision of one cross linked arm and a subsequent recombination of homologous duplexes^{16,17}. When the excision repair capacity of FA lymphocytes was tested, their level of DCMC- and MMC-stimulated unscheduled DNA synthesis was comparable with that of normal cells. Thus if a repair mechanism like that proposed for cross links in bacteria can apply to human cells, the FA cells could be defective in the later step of the sequential repair process. A simple application of this repair process, especially an excision step by endonucleolytic incision cannot, however, easily explain the repair of cross links in mammalian cells.

With respect to differing sensitivity of cell stage to chromosome breakage, xeroderma pigmentosum cells respond to MMC as the repair-proficient cells while they are deficient in unscheduled DNA synthesis induced by MMC (my unpublished data). Similarly, rat kangaroo cells show a chromosome response of the repair-proficient type to sulphur mustard¹² in spite of their unresponsiveness, in terms of unscheduled DNA synthesis, to ultraviolet light¹⁸. Furthermore, cross links induced by sulphur mustard can be repaired in mouse L cells¹⁹ which have very little ability to excise ultraviolet damage²⁰. These observations suggest that, unlike bacteria in which sensitivity to MMC, sulphur mustard and ultraviolet light is commonly associated with the *uvr* gene^{21,22}, mammalian cells can repair cross links by a process independent of the excision ability responsible for the repair of damage induced by ultraviolet light. The identification of the site of the primary defect in FA cells awaits elucidation of such a repair process. My results, however, strongly suggest that FA cells are defective in a biological system which constitutes an essential step in the repair of DNA interstrand cross links.

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Organisation and evolution of *Drosophila virilis* heterochromatin

CENTRIC heterochromatin in *Drosophila* metaphase chromosomes is organised into large blocks which show various degrees of condensation in prometaphase¹ and various degrees of decondensation when living cells are Hoechst treated². By sequential staining with the fluorescent dyes Hoechst and quinacrine, three different types of blocks are distinguishable in *D. virilis*. Each block seems to contain only one DNA satellite and similar staining blocks, along with their respective satellite, appear or disappear as units during speciation; this was first suggested from a study of *D. hydei* sibling species¹ and here confirmed for the *D. virilis* group.

The *virilis* group species in Fig. 1 are phenotypically almost indistinguishable and interspecific crosses, excepting with *D. ezoana*, produce fertile offspring³. Maps of their polytenised euchromatin are identical except for inversions and translocations^{3,4}. Heterochromatin differs drastically within the group as evidenced by acrocentric fusions, heterochromatin elimination and the presence or absence of various satellite DNAs in centromeric heterochromatin (Fig. 1).

Sequential staining of *D. virilis* metaphase chromosomes with Hoechst, quinacrine and Giemsa reveals three different types of heterochromatin blocks enabling one to identify each chromosome by the blocks it possesses (Fig. 1). The remaining species lack H⁺ or Q⁺ blocks so their chromosome arms, being of similar size, cannot usually be identified. In interspecific hybrids, however, such as the *D. virilis* × *D. texana* hybrid of Fig. 2, the euchromatic arms of *D. texana* chromosomes are often somatically paired with homologous and identifiable arms of *D. virilis* chromosomes. Here, by comparing the fluorochromatically differentiated heterochromatin of homologous arm pairs from different species, one sees that *D. texana* has 65% less heterochromatin than *D. virilis* and lacks block α₁. The *virilis* group's heterochromatin is thus organised into distinct blocks and similar blocks are lost or gained during speciation (Fig. 1).

In *D. virilis*, there seems to be a 1:1 relationship between the three different fluorochromatic blocks and the three different DNA satellites which is based on indirect evidence from various sources. *D. melanogaster* shows five satellites which are located in centric heterochromatin⁵⁻⁷. Some of these satellites, as localised by *in situ* hybridisation, are confined to only one or a few chromosomes⁷. Four satellites are homogeneous non-interspersed tandem repeats of a small oligonucleotide; the repeats are at least 600 kilobases (kb) long⁷. *D. virilis* satellites present a similar story. ³H-RNA complementary to satellite I hybridised *in situ* to heterochromatin of all chromosomes⁸; however, since heterologous satellite hybrids have melting points close to the *in situ* annealing conditions used⁹ and autoradiographic resolution is limiting, the exact location of satellite I was not determined. Sarcosyl lysates of *D. virilis* cells, having

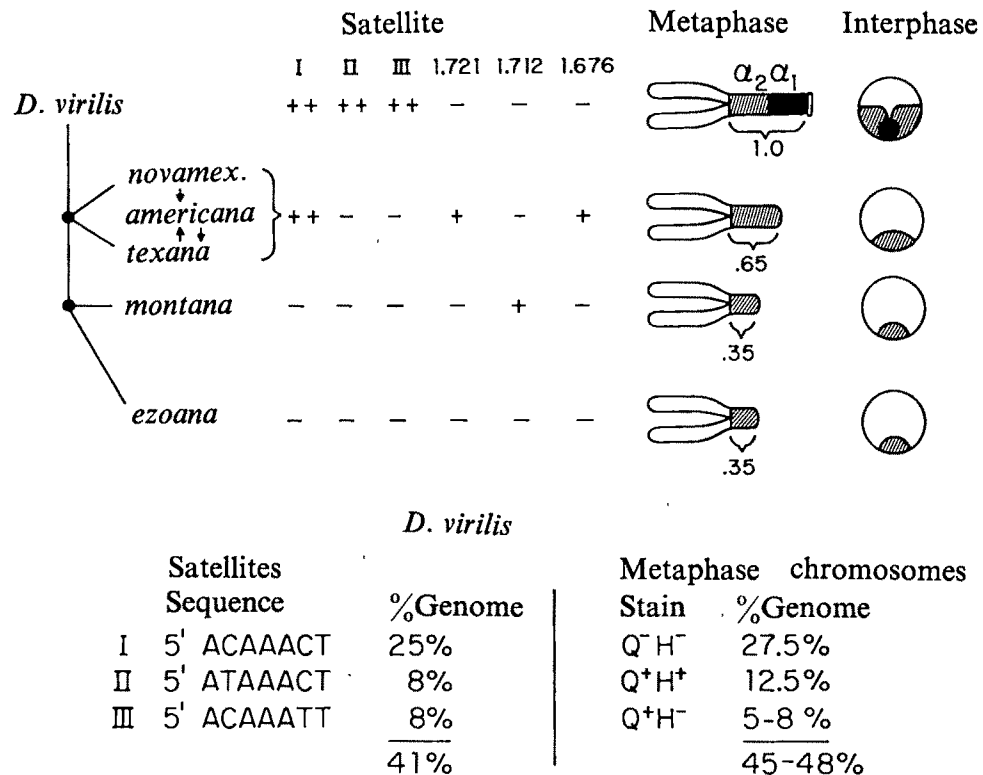


Fig. 1 Phylogenetic relations of the *D. virilis* group^{3,4} are presented as family tree. Species which evolved from *D. montana* and *D. ezoana* are not included. Satellites common to each species^{10,11} are indicated. Minor satellites, which comprise less than 5% of the genome, are designated by their buoyant density in neutral CsCl. One stylised chromosome arm and an interphase nucleus from female larval neuroblasts of each species are presented. Alpha heterochromatin of each *D. virilis* chromosome is composed of two fluorochromatic blocks. The distal α_2 block is Q⁻H⁻ while the proximal α_1 block is Q⁺H⁺ for chromosomes X, 2 and 4, Q⁺H⁻ for chromosome 3, and Q⁻H⁻ for chromosome 5 (ref. 1). Chromosomes from the remaining five species show less heterochromatin and no Q⁺ or H⁺ blocks of heterochromatin. All six species have the same amount of euchromatin²; the amount of heterochromatin is expressed as prometaphase chromosome length of heterochromatin/length euchromatin where the amount of euchromatin is normalised to 1.0. For example, *D. americana* and *D. texana* each have an average of 0.65 units of heterochromatin associated with each euchromatic chromosome arm. *D. novamexicana* has approximately the same amount of heterochromatin as these two previous species even though most of it is associated with one chromosome pair. Centric heterochromatin aggregates into a chromocentre in diploid (right hand column) and polytene interphase nuclei. *D. virilis* interphase nuclei are particularly interesting because the heterochromatic chromocentre is divided into three regions possibly representing the three different satellites²⁴. The central region is H⁺ and partially separates the two Hoechst-dull regions. The other *virilis* group species lack H⁺ or Q⁺ material in their chromosomes and interphase nuclei. The size of their interphase chromocentres parallels their content of metaphase heterochromatin. The table shows the three heptanucleotide sequences which, when tandemly repeated, constitute the three *D. virilis* satellites^{10,11}. The percentage of the diploid genome each satellite comprises is shown next to its base sequence. The percentage prometaphase chromosome length devoted to each permutation of Hoechst-quinacrine brightness is from previous data¹ and is arranged to correspond to the satellite data. Estimates of percentage genome devoted to a particular 'type' of chromatin are rather inexact because of differential chromatin condensation during various stages of mitosis.

DNA molecules averaging at least 90 kb in length, show excellent satellite separation after analytical buoyant density ultracentrifugation^{8,10,11}. Thus, similar to the findings with *D. melanogaster* satellites, one heptanucleotide is repeated many times without interdigitation and forms almost pure repeating heptanucleotide tracts at least 90–180 kb long. By extending these molecular weight findings a little, one can imagine a pure repeating heptanucleotide tract 15,000 kb long. This is just enough DNA to fill one fluorochromatic block (one-quarter of a chromosome) (Fig. 3). Each block, being homogeneous in its DNA, would show a homogeneous fluorescence after quinacrine or Hoechst staining and this is indeed seen¹. There are three satellites in heterochromatin and three differently staining blocks of heterochromatin. The amount of each satellite and amount of heterochromatin showing a particular quinacrine-Hoechst permutation can be arranged to correspond quite well (Fig. 1). This correspondence ascribes satellite I to the Q⁻H⁻ blocks. Satellite I, having the highest GC content of the three satellites, would be predicted to correspond to the most weakly fluorescing blocks¹²⁻¹⁴ as it does. Satellites II and III are absent from *D. americana*, *D. texana* and *D. novamexicana*^{10,11} as are their ascribed Q⁺H⁺ and Q⁺H⁻ blocks (Figs 1 and 2). In hybrid cells containing somatically paired chromosomes, this loss seems to be a simple excision of the Q⁺H⁺ or/and Q⁺H⁻ block (Fig. 2). When comparing the sibling species *D. hydei*, *D. neohydei* and *D. eohydei*, the same relationships hold^{11,16}. Here

the presence of fluorochrome bright and dull blocks parallel the presence of AT-rich and GC-rich satellites, respectively. Here again the polytene banding patterns are almost identical, only the non-polytenised centric heterochromatin is different between species. The lack of Q⁺ or H⁺ material in *D. americana*, *D. texana* and *D. novamexicana* cannot be due to lack of a specific protein which confers fluorochrome-bright properties to heterochromatin because the staining properties of each chromosome in *virilis* × sibling species hybrids remains unchanged. Finally, the absence of satellite I in *D. ezoana* and *D. montana* is paralleled by an extreme loss of Q⁻H⁻ heterochromatin. The simplest model of chromosome organisation in accord with all existing data is that each fluorescent block of centric heterochromatin in *D. virilis* contains one continuous repeating DNA molecule consisting of a single heptanucleotide repeated about two million times which, when coated with protein, condensed and packaged, forms a homogeneous appearing block of centric heterochromatin with fluorescent properties determined by the heptamere it contains (Fig. 3).

In chromosomes of the *virilis* group, certain alterations of heterochromatin occurred, were selected for and subsequently fixed during evolution. Knowing these alterations, we can now make some statements concerning the organisation and evolution of heterochromatin in *Drosophila*.

(1) Heterochromatin is more taxonomically divergent than external morphological characteristics^{1,15}; one can more easily

identify the fly by looking at its metaphase chromosomes than by looking at the fly itself. Speciation has proceeded with the loss or gain of large heterochromatin blocks (Fig. 1) although the euchromatic arms have remained relatively unchanged^{3,4}.

(2) The 2-3 metacentric in *D. texana* (Fig. 2) and a similar X-4 metacentric in *D. americana* were thought to represent simple Robertsonian fusions of two *D. virilis* acrocentrics^{10,11} but this is obviously not true (Fig. 2). If these metacentrics actually evolved from the present *D. virilis* chromosomes, then fusion was preceded by, or concomitant with, loss of α_1 blocks, loss of the short Q^+H^- right arms and relocation of the *virilis* centromeres into Q^-H^- heterochromatin. Although the exact process of chromosome fusion that actually took place remains unknown, it was irreversible and more complex than the simple, reversible Robertsonian fusions which occur between telocentric chromosomes during speciation in mice.

(3) Satellite DNA cannot account for all the heterochromatin in any of the *virilis* group species (Fig. 1). This is most evident in *D. ezoana* which lacks satellite but, like all *virilis* group species, has some heterochromatin in each chromosome. The complete absence of satellite DNA in many of these species^{10,11}, even in alkaline CsCl gradients, argues against the presence of a cryptic satellite. Thus, each Q^-H^- block in *D. virilis*, *americana*, *texana* and *novamexicana* must be composed of two DNAs, satellite I and non-satellite, and two different Q^-H^- blocks which cannot be distinguished by fluorometric means. The non-

satellite block would correspond to the heterochromatin remaining *D. ezoana* and *D. montana*. Being ubiquitous within the group, this block must contain the heterochromatic genes and about the euchromatin-heterochromatin junction (Fig. 3). The heterochromatin of the *D. melanogaster* X is similarly Q^-H^- (ref. 1), lacks satellite DNA⁷ and contains the sex-linked heterochromatic genes¹⁶.

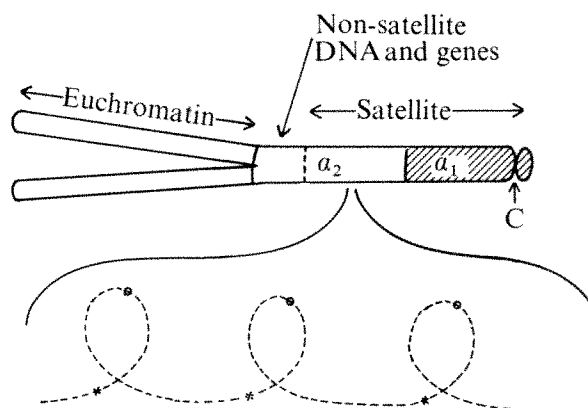


Fig. 3 The single DNA molecule, which runs the length of the chromosome²⁰, shows three successive levels of DNA organisation in *D. virilis* heterochromatin. The basic level is a tandemly repeated heptanucleotide (—)^{10,11}. The second level, a 2-30+ repeating unit, was detected in *D. melanogaster*^{17,18} as equally spaced restriction endonuclease sensitive sites (*, ○) similar to bovine satellite I DNA¹⁹. Lastly, the 2-30-kb repeats are packaged into large fluorochromatic blocks which contain about 17,000 kb = one-quarter of a chromosome. The α_1 block and the short arm opposite the centromere (C) are fluorochrome bright and contains satellites II or III. The dull α_2 block contains satellite I in its proximal region and non-satellite DNA in its distal region. Although Peacock *et al.*⁵ reported satellite DNA in euchromatic polytene bands 21CD of *D. melanogaster*, I find this hybrid to have a 15 °C lower melting point than centromeric hybrids. Thus, 21CD DNA differs from centromeric satellite⁸ and euchromatic regions may indeed be free of true centromeric satellite DNA.

(4) Similar blocks of heterochromatin and similar satellites appear or disappear during speciation even though these similar blocks or satellites are usually on many different chromosomes. This may imply selection for interchromosomal interaction of like blocks of heterochromatin. In interphase nuclei, the heterochromatin aggregates into a chromocentre wherein similar blocks of heterochromatin aggregate on to themselves to the exclusion of others (Fig. 1). This aggregation also seems to direct somatic non-disjunction in colcemid-anaphase nuclei of *D. virilis*¹. There are three types of chromosome-chromosome pairing in *Drosophila* somatic nuclei: somatic pairing of identical euchromatin, centric aggregation of identical or similar centric heterochromatin and ectopic pairing of polytene bands which are often morphologically¹⁷ or chemically^{18,19} similar. All three pairings involve two chromosome segments which contain identical or suspiciously similar DNA. Selection for interchromosomal pairing of heterochromatin would explain the simultaneous appearance or disappearance of quasi-dispersed satellites during speciation.

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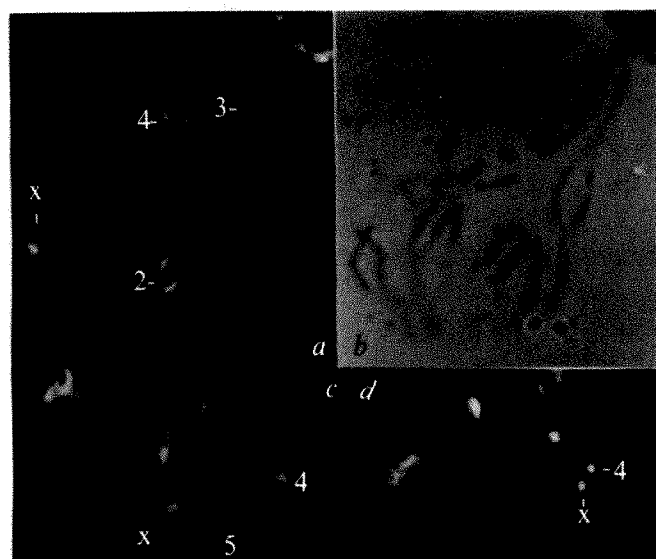


Fig. 2 Neuroblast prometaphase chromosomes from hybrid *D. virilis* × *D. texana* third instar larvae sequentially stained with Hoechst, quinacrine and Giemsa to distinguish each chromosome. Neuroblast metaphase preparations were stained with Hoechst¹, destained (three overnight rinses in pyridine), stained with quinacrine and finally stained in Giemsa. The pyridine removal method enables one to initially scan a Hoechst-stained slide when chromosomes are brightest and subsequently stain with the dimmer (quinacrine) fluorochrome. *a*, *c* and *d*, Hoechst stained; *b*, Giemsa stained from *a*. *D. virilis* chromosomes are indicated by number. Somatic pairing of homologous euchromatic arms allows one to unambiguously identify each *D. texana* arm because the homologous *D. virilis* arm is identifiable. For example, in (*a*), the upper arm of the 2-3 metacentric of *D. texana* pairs with the Q^+H^- *D. virilis* chromosome 3. The lower arm pairs with the Q^+H^+ *D. virilis* chromosome 2. Even though it is difficult to distinguish between the two Q^+H^+ chromosomes 2 and 4 in *D. virilis*, one can easily do this in such hybrids where *D. virilis* 2 must pair with one arm of the *D. texana* 2-3 metacentric. This sibling species-somatic pairing method should be quite useful for chromosome identification in many other dipteran groups. Alpha heterochromatin condenses earlier than euchromatin^{1,8} and is most apparent in Giemsa stained preparations of very early prometaphase (*b*). The Q^+H^+ blocks of α_1 heterochromatin condense even earlier than α_2 heterochromatin¹ so the H^+ blocks in early prometaphase (*a*) appear relatively smaller than the H^+ blocks in late prometaphase (*c* and *d*). *D. texana* chromosomes look like *D. virilis* chromosomes which have lost the α_1 block of heterochromatin.

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Segregation of RD-114 and FeLV-related sequences in crosses between domestic cat and leopard cat

TYPE C viruses of the RD-114 (ref. 1) group have been isolated, either spontaneously or after chemical induction, from cell cultures of the domestic cat (*Felis catus*)^{2–4}. Nucleic acid sequences related to the RD-114 genome are in the DNA of all domestic cats^{5–8}. Thus these viral genomes are transmitted vertically from parent to offspring as integral components of cat cellular DNA. Although the family Felidae consists of closely related animals, only four *Felis* species have been found to contain RD-114-related sequences. These include the domestic cat, the European wildcat (*F. sylvestris*), the sand cat (*F. margarita*), and the jungle cat (*F. chaus*); other members of the Felidae lack nucleic acid sequences related to RD-114 (ref. 9). The observation that RD-114 is partially related to the endogenous baboon type C viruses^{10–12} and that sequences related to RD-114 are found in the cellular DNA of all Old World monkeys led to the postulate that this group of viruses originated from an endogenous primate type C virus¹³ transmitted horizontally to the germ line of ancestors of certain *Felis* species during the Pliocene or early Pleistocene somewhere in the region of the Mediterranean basin⁹.

A second distinct group of type C viruses, the feline leukaemia viruses (FeLV), also has been isolated from domestic cats¹⁴. Although FeLVs are horizontally transmitted among domestic cats, genes partially related to the RNA genome of FeLV are found in *F. catus* DNA¹⁵ and in the DNA of the other species of Felidae which contain RD-114 related nucleic acid sequences¹⁶. Viruses of the FeLV group are also postulated to have been transmitted to an ancestor of these *Felis* species, but to have originated from a rodent rather than a primate source¹⁶.

The leopard cat (*F. bengalensis*) is a spotted wildcat found throughout South-east Asia which lacks RD-114 and FeLV-related DNA sequences (RD[–], FL[–])⁹. Since leopard cats produce viable offspring when bred with domestic cats (RD⁺, FL⁺), we studied the segregation of both sets of virogenes in F₁ hybrids and in the progeny of a backcross to the RD[–], FL[–] parent. The cellular DNA of the F₁ hybrids contains half the number of copies of each set of sequences. The RD and FL virogenes segregate together in the backcrossed animals in a manner consistent with their localisation at a single chromosomal site.

The reassociation kinetics obtained by hybridising ³H-DNA transcripts of viral RNA to cellular DNA can be used to estimate relative gene frequencies by determination of half *C*₀*t* values (the midpoint of the renaturation curve)¹⁷. The number of gene copies can also be estimated by plotting reassociation kinetics as the reciprocal of the fraction of unhybridised ³H-DNA against *C*₀*t* (Wetmur–Davidson plot)¹⁸. In such plots, the slope is proportional to the number of copies of those sequences measured. Using RD-114 ³H-DNA probes,

multiple copies of virus-related sequences can be detected in the cellular DNA of stray domestic cats, domestic cats reared in a germ-free environment (Merck, Sharp and Dohme, West Point, Pennsylvania) and in European wildcats (*F. sylvestris*) (Fig. 1a and Table 1). The *C*₀*t*_{1/2} values ranged from 120 to 170. In contrast, the cellular DNA of the leopard cat completely lacks RD-114 related sequences. The *C*₀*t*_{1/2} values for the self-annealing of non-repetitive domestic cat cellular DNA, and for the hybridisation of the ³H-DNA RD-114 probe to the DNA of a canine thymus cell line infected with RD-114, ranged from 1,800 to 2,000 (Fig. 1, Table 1). Given that *C*₀*t*_{1/2} values of 1,800–2,000 are obtained with genes present in a single copy per haploid genome, domestic cat and European wildcat cellular DNAs contain 10–13 copies of RD-114-related sequences per haploid genome. Since these copies represent a family of diverging gene sequences only partially related to one another¹⁹, the calculated number of copies may be an underestimate^{20,21}.

Leopard cat males were mated to domestic cat females and the F₁ hybrids studied. These DNAs contain a complete complement of sequences related to the RD-114 probe, but only half the number of copies present in the domestic cat parent (Fig. 1a). The *C*₀*t*_{1/2} values (275–350) represent, as a minimum estimate, five to seven virogenic copies per haploid genome. Two kittens obtained from an F₁ hybrid female backcrossed to the leopard cat (Fig. 2) were also studied. Figure 1a shows that kitten No. 1 contains all the RD-114-related information, but only half the number of copies (*C*₀*t*_{1/2} 280), like the F₁ parent, whereas kitten No. 2 (from the same litter) lacks RD-114-related DNA sequences like its leopard cat parent. These results suggest that the multiple copies of RD-114 re-

Table 1 Segregation of RD-114 and FeLV type C viral sequences in various cats

Cats*		RD-114		FeLV	
		<i>C</i> ₀ <i>t</i> _{1/2} †	Average no. viral copies‡	<i>C</i> ₀ <i>t</i> _{1/2}	Average no. of viral copies
Domestic cat	No. 1	130	12–13	230	8–9
	No. 2	120	12–13	260	7–8
	No. 3	170	10–11	280	7–8
CCC clone 6§		150	10–11	250	7–8
European wildcat		140	11–12	210	8–9
F ₁ hybrid	No. 1	350	5–6	550	3–4
	No. 2	330	5–6	400	4–5
	No. 3	275	6–7	500	3–4
	No. 4	300	6–7	450	3–4
Backcross	No. 1	280	6–7	520	3–4
	No. 2	—	0	—	0
Leopard cat	No. 1	—	0	—	0
	No. 2	—	0	—	0
Cell lines					
RD-114/FCf2Th					
clone 10§		1,800	1	—	0
FeLV/FCf2Th§		—	—	1,900	1

* Cellular DNA was extracted from various organs (spleen, liver, kidney, lung) and hybridised to RD-114 and FeLV ³H-DNA as described in Fig. 1. Domestic cats Nos 2 and 3 were from the germ-free colony of cats at Merck, Sharp and Dohme; animals from this colony have never been found to be positive for infectious feline leukaemia virus. F₁ hybrid cats Nos 1, 2 and 3 and 4 belong to three separate litters.

† *C*₀*t*_{1/2} values represent the midpoint of the reannealing curves¹⁷.

‡ The approximate number of copies per haploid genome of sequences related to either RD-114 or FeLV were estimated from reciprocal plots (Fig. 1). The number of copies is determined by the ratio of the slope of each line to the slope of the line described by the reassociation of non-repetitive domestic cat cellular DNA (*C*₀*t*_{1/2} = 1,800–2,000; see also ref. 19). In the case of RD-114, where two sets of viral sequences can be detected in cellular DNA, the number of copies listed is the average of the two populations.

§ CCC clone 6 is from a continuous line of domestic cat kidney fibroblasts and is not releasing type C virus^{2,3}, and RD-114/FCf2Th and FeLV/FCf2Th are a dog thymus cell line infected, respectively, with RD-114 (ref. 1) or with the helper virus from the Gardner–Arnststein²³ strain of feline sarcoma virus.

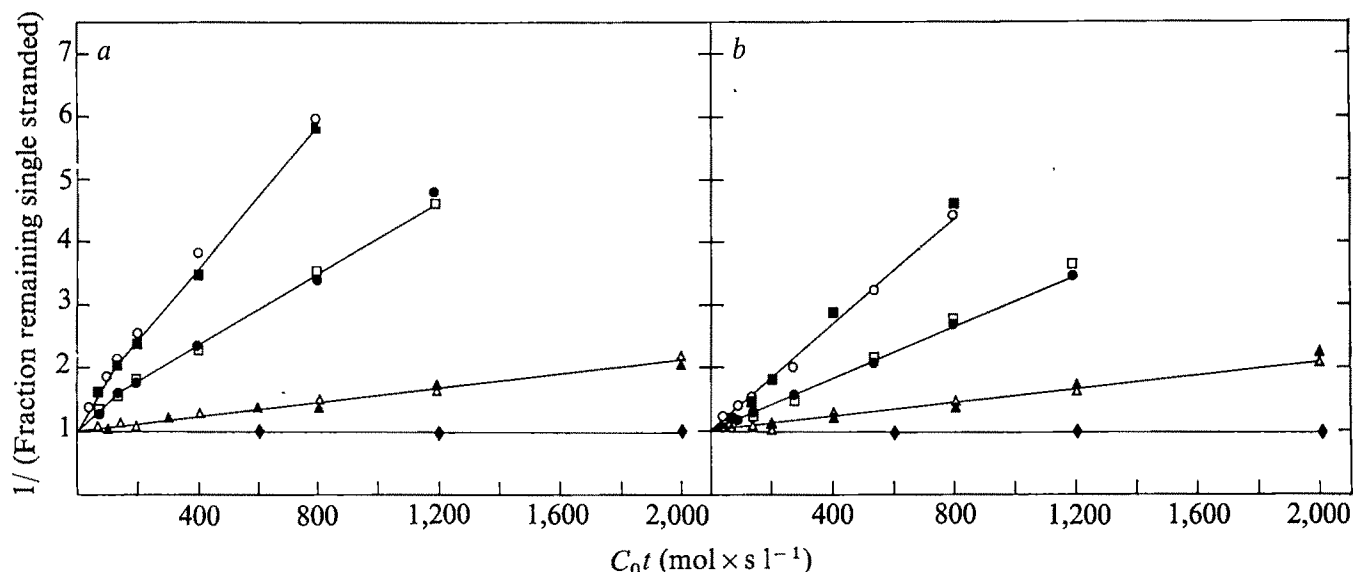


Fig. 1 Analysis of reassociation kinetics by the method of Wetmur and Davidson¹⁸ of RD-114 and FeLV ³H-DNA probes to cat cellular DNAs, and of domestic cat unique sequence cell DNA self-association. The ³H-thymidine-labelled DNA probes were synthesised from detergent-disrupted type C virus in the presence of actinomycin D as described²⁷. The specific activity of the ³H-DNA was 1.7×10^7 c.p.m. μg^{-1} . The ³H-DNA probes contained 60–66% of their respective 70S viral RNA sequences at a ³H-DNA:³²P viral RNA molar ratio¹⁰ of 2.0. Cellular DNA was extracted from tissues and cell lines as described¹⁰. All DNAs were treated sonically so as to yield a mean size of 6–8S (the size of the ³H-DNA probes)¹⁰. DNA:DNA hybridisations were incubated at 65 °C in reaction mixtures containing 0.01 M Tris, pH 7.4, 0.75 M NaCl, 2×10^{-3} M EDTA, 0.05% sodium dodecyl sulphate, 30,000 to 40,000 c.p.m. of ³H-DNA and 2–4 mg of cellular DNA per ml. Hybridisations were started by heating the mixtures to 100 °C for 10 min, cooling on ice to 4 °C and incubating at 65 °C. At various times, 0.025 ml portions were removed and frozen at –80 °C until digested with the single-strand specific nuclease, S₁, as described²⁷. C_0t values (C_0 is the concentration of cellular DNA in mol l^{-1} and t is the time in s) were calculated as suggested by Britten and Kohne²⁸ as $(A_{260} \text{ ml}^{-1})/2 \times h$, and corrected to a monovalent cation concentration of 0.18 M (ref. 29). a, Annealing of RD-114 ³H-DNA probe to DNA extracted from: ○, domestic cat No. 3; ■, domestic cat No. 1; ●, F₁ hybrid No. 4; □, backcrossed kitten No. 1; ▲, a dog thymus cell line infected with RD-114, and ◆, leopard cat and backcrossed kitten No. 2. Comparable data were obtained with DNA probes prepared from the domestic cat type C virus, CCC^{2,3}, and with the FS-1 virus isolated from European wildcat cells³⁰. Δ, Self-association of non-repetitive domestic cat cellular DNA. ³H-thymidine-labelled domestic cat cellular DNA was isolated by removing the highly reiterated sequences that anneal by a C_0t of 800 (approximately 40% of the total DNA) by fractionation on hydroxyapatite¹⁰. This ³H-thymidine-labelled DNA (5×10^6 c.p.m. μg^{-1}) was then hybridised to total domestic cat cellular DNA. b, Annealing of FeLV ³H-DNA probes (Rickard strain)²² to DNA extracted from: ○, domestic cat No. 2; ■, domestic cat No. 1; ●, F₁ hybrid No. 3; □, backcrossed kitten No. 1; ▲, a dog thymus cell line infected with FeLV, and ◆, leopard cat and backcrossed kitten No. 2. Comparable data were obtained with the Gardner-Arnstein²³ strain of FeLV grown in a canine thymus cell line (FCf2Th). Δ, Self-association of unique sequence domestic cat cellular DNA. Domestic cat cellular DNA contains at least two distinct sets of RD-114-related sequences which are present in different reiteration frequencies. The F₁ hybrid and backcrossed kitten No. 1 have approximately half the number of copies of each set of related sequences. While only one set of virogyne sequences can be detected with the FeLV probe (Fig. 1b), the F₁ hybrid and backcrossed kitten No. 1 again are shown to contain half the number of copies as that found in the domestic cat parent.

lated sequences are located together at one (or relatively few) chromosomal sites.

The same cats were examined for FeLV-related genes using ³H-DNA probes prepared from various strains of FeLV^{22,23}. No cross-hybridisation between these probes and RD-114 is detectable^{15,24}. The results parallel exactly the data obtained with transcripts of RD-114 RNA. The domestic cat parent contains multiple copies of FeLV-related virogenes whereas the leopard cat parent lacks these sequences. The F₁ hybrids contain half the number of copies. Backcrossed kitten No. 1 has the same number of virogyne copies as its parent (the F₁ hybrid) although its littermate has no detectable sequences related to FeLV.

Table 1 summarises the hybridisation data; four clear classes of DNAs are evident. The first consists of animals that contain full complements of RD-114 and FeLV-related gene sequences. These cats contain both sets of virogyne sequences reiterated a comparable number of times, although there may be fewer FeLV-related copies. The animals in the second class, including all the F₁ hybrids and one of the backcrossed kittens, contain half the virogyne complement of the RD⁺, FL⁺ parents. A third class contains sequences that anneal to the viral probe with reassociation kinetics comparable with that seen for the association of the most slowly reannealing cellular DNA sequences and therefore probably contains one viral copy. This includes cloned heterologous cell lines producing high titres of either RD-114 or FeLV viruses. The fourth class completely lacks sequences related to either RD-114 or FeLV and includes the leopard cats and one of the backcrossed kittens.

If the multiple copies of RD-114 and FeLV-related sequences present in cat DNA had become physically separated from one another after their entry into the cat germ line⁹ they would not all have segregated together. The data rule out the possibility

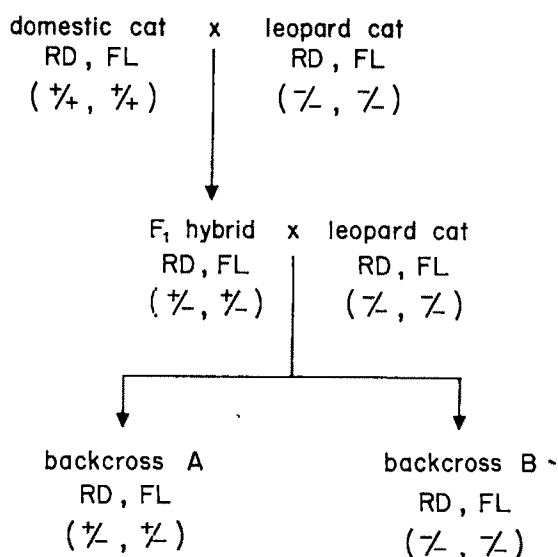


Fig. 2 RD-114 and FeLV type C viral genotypes of the various cats studied.

that each of the multiple copies of RD-114 or FeLV-related virogenes occurs on a different linkage group, as well as models of non-chromosomal inheritance of the multiple viral copies.

Since only certain *Felis* species contain RD-114 and FeLV-related sequences in their DNA, we propose that both classes of viruses were acquired by cats subsequent to their major radiation, most likely in the Pliocene, and that both sets of sequences have been perpetuated in the germ line^{9,18}. The multiple virogenes copies presumably arose as a result of gene duplication and/or unequal crossing-over after infection. The presence of multiple copies of both sets of virogenes in cat cellular DNA seems to be a general property of endogenous mammalian type C viruses; mouse, rat, hamster, pig and baboon DNAs also contain similar numbers of copies of their respective endogenous viruses¹⁹.

The genetic crosses described here provide a new approach to the study of the evolution of multiple gene systems. The virogenes sequences can be considered as one of the group of moderately repetitive sequences such as the genes for 5S RNA²⁵, histones²⁶ and feather keratin²⁰. The existence of natural populations of animals that either lack or contain DNA sequences related to both RD-114 and FeLV, and the ability of these cats to interbreed permits the study of the physiological and potentially pathological role of each of these genetically transmitted gene sequences. Hybrid animals containing half the number of virogenes copies and virogenes-negative cats should allow the study of the effects of gene dose on susceptibility and resistance to diseases mediated by both groups of type C viruses.

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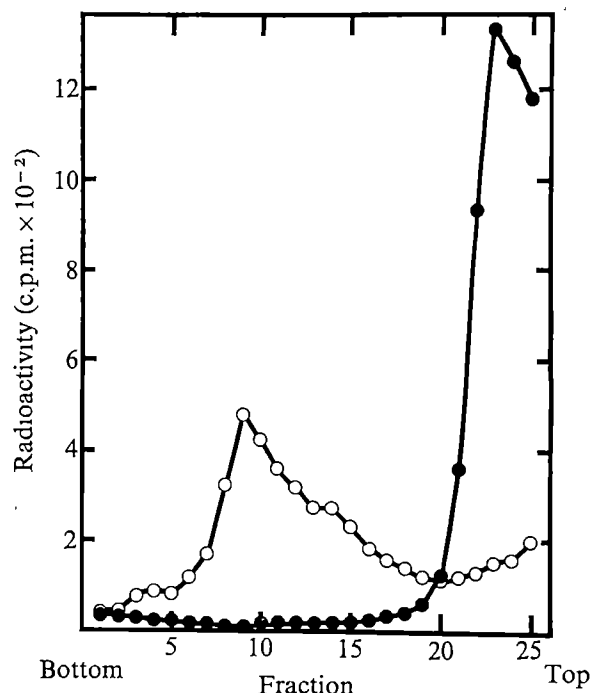
Identification of heat-dissociable RNA complexes in two porcine coronaviruses

THE coronavirus genome has been shown to comprise single-stranded RNA^{1,2}. Examination of the viral nucleic acid synthesised by pig kidney cells infected with transmissible gastroenteritis virus (TGEV) suggested that several molecular species, ranging in size between 18 and 28S, were involved in the viral replicative cycle³; similarly Tannock found a wide variation in the size of RNA molecules extracted from avian infectious bronchitis virus (IBV) by a phenol-sodium dodecyl sulphate (SDS) method². Extraction of IBV RNA by 1% SDS at 60 °C has, however, revealed a single component of molecular weight 9×10^6 corresponding to 60S by electrophoresis through 2.2% polyacrylamide gels⁴.

We have examined the RNA extracted from purified preparations of TGEV and a second porcine coronavirus—haemagglutinating encephalomyelitis virus (HEV)—and have found a 60-70S RNA component which dissociates into 35S and 4S material on heating above 60 °C in a way that closely resembles the genome of the oncogenic RNA viruses.

We had observed that treatment of purified TGEV with 1% SDS at 20 °C disrupted the virions and liberated a high molecular weight complex containing the RNA. On the assumption that this complex might comprise the hitherto undetected ribonucleoprotein, we extracted the material from TGEV preparations radioactively labelled with ³H-uridine or with ³H-leucine to determine which structural polypeptide was associated with the complex. As is shown in Fig. 1, however, the fast moving RNA complex has no detectable protein associated with it, while polyacrylamide gel analysis of the radioactivity remaining near the top of

Fig. 1 Rate zonal sedimentation of TGEV after treatment with 1% SDS at 20 °C. TGEV was grown in secondary pig thyroid cell (APT/2) cultures in the presence of 5-³H-uridine (○) or 4,5-³H-leucine (●) and purified by sucrose gradient centrifugation as described previously⁵. After treatment with 1% SDS at 20 °C for 15 min, each preparation was layered over a 6-ml 15-30% (w/w) sucrose gradient and centrifuged at 250,000g for 2 h in a swing-out rotor. The gradients were fractionated by siphon and total radioactivity was determined for each fraction.



the gradient demonstrated the presence of viral structural polypeptides⁵.

Extraction of RNA from ³H-uridine-labelled TGEV by 1% SDS at 20 °C followed by electrophoresis through gels containing 2% polyacrylamide and 0.6% agarose revealed a homogeneous band of high molecular weight RNA and a very small amount of radioactivity in the area of the marker dye, corresponding to approximately 4–7S (Fig. 2a). Similar extractions were performed on replicate virus samples using 1% SDS at 40, 60, 80 and 100 °C and the released RNA was electrophoresed as before. The electrophoretograms illustrated in Fig. b–e showed that the mobility of the major RNA band increased slightly as the extraction temperature was raised to 60 °C and, while the homogeneity of the leading edge of the band decreased somewhat, there was no obvious change in the 4S–7S region. As the temperature was increased through 80 to 100 °C, however, the radioactivity became associated with a broader band of RNA, whose mobility was at least twice that of the RNA extracted at lower temperatures, and there was an increase in the amount of RNA in the 4S region of the gel.

This apparent "melting" of the high molecular weight complex into smaller components with the liberation of 4S

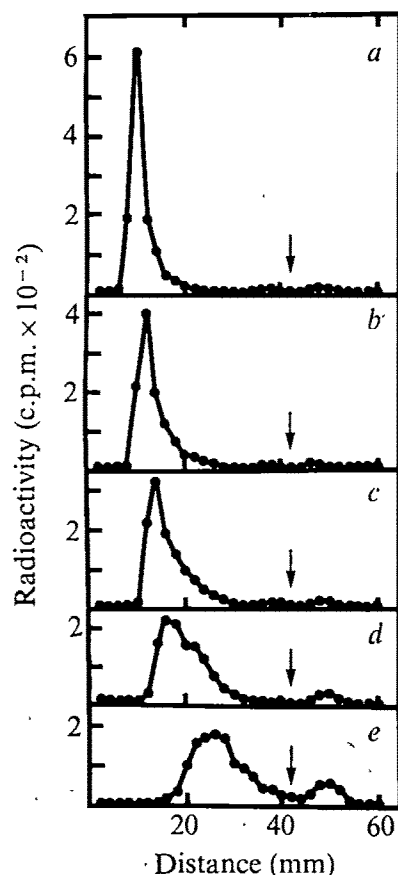


Fig. 2 Gel electrophoresis of TGEV RNA following extraction in 1% SDS at various temperatures. TGEV was grown in APT/2 cultures in the presence of ³H-uridine and purified by sucrose gradient centrifugation. The preparation was suspended in L buffer (36 mM 2-amino-2-hydroxymethylpropane-1,3-diol (Tris); 30 mM NaH₂PO₄; 1 mM ethylenediaminetetraacetic acid (EDTA); pH 7.8) and SDS was added to 1% at 20 °C. The suspension was divided into five parts and each part was held in a water bath at 20 (a), 40 (b), 60 (c), 80 (d) or 100 °C (e) for 30 min. After cooling, sucrose was added to 5% (w/w) and bromophenol blue was added as a marker dye, each sample was layered over a 6 × 60 mm 2% (w/v) polyacrylamide/0.6% agarose gel⁶ and electrophoresed for 1 h at 8 mA per gel using L buffer containing 0.2% SDS. The gels were sliced into 1-mm fractions and the radioactivity of each slice was determined in a toluene-Triton X-100 scintillant. The location of the marker dye was estimated by eye and is represented by the arrows.

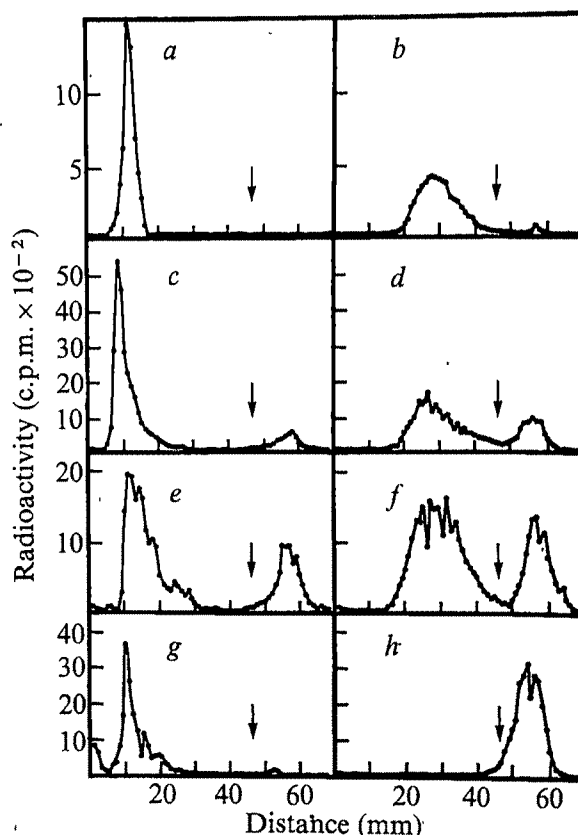


Fig. 3 Gel electrophoresis of RNA from RSV, TGEV, and HEV. TGEV and HEV were grown in APT/2 and primary pig kidney cell cultures respectively in medium containing ³H-uridine. Both viruses were purified by sucrose gradient centrifugation and suspended in L buffer. After 24 h at 4 °C or, in the case of the virus used in (g) and (h), after 20 d at 4 °C, SDS was added to 1% and the preparations were held at 20 or 100 °C for 30 min. Purified 60–70S RNA from RSV was held in 10 mM NaCl; 8 mM Tris; 7 mM NaH₂PO₄; 1 mM EDTA; 1% SDS; pH 7.8 at 20 and 100 °C for the same length of time. The preparations were cooled to 20 °C, sucrose and bromophenol blue were added and electrophoresis and fractionation were conducted as described for Fig. 2, using 6 × 70 mm gels. RSV RNA held at 20 (a), and 100 °C (b); TGEV extracted at 20 (c), and 100 °C (d) after 24 h at 4 °C; HEV extracted at 20 (e), and 100 °C (f) after 24 h at 4 °C; TGEV extracted at 20 (g) and 100 °C (h) after 20 d at 4 °C. The arrows denote the location of the marker dye.

RNA at temperatures above 60 °C closely resembles the findings for the oncogenic RNA viruses⁷. To determine the size of the coronaviral RNA components and their similarity to oncornaviral RNA, we compared the mobilities of RNA extracted from TGEV and HEV at 20 and 100 °C with those of purified Rous sarcoma virus (RSV) RNA held at the same temperatures. Figure 3a–f shows that, by this method, the RNA complexes extracted from the two coronaviruses are indistinguishable in size from the 60–70S component of RSV RNA and that, after heating, the TGEV and HEV RNA components are comparable in size to the RSV 35S RNA. There seems to be more heterogeneity in the coronaviral 35S RNA band than in the RSV equivalent and this holds true regardless of the time of the labelling period. Comparison of RNA extracted from TGEV labelled with ³H-uridine 0–4, 4–8, 8–12, 12–16 or 0–20 h after infection shows that, although the total radioactivity incorporated varied with the time of labelling period, the overall shape of the RNA curves after extraction at 20 and 100 °C were similar. This difference in heterogeneity may be due partly to the fact that we were comparing extracts from whole HEV and TGEV with purified 60–70S RNA from RSV, but it may also be caused by partial degradation of the RNA within the virion. That this undoubtedly occurs is demonstrated by the electrophoretograms illustrated in Fig. 3g–h, which were all derived

from TGEV that had been held at 4 °C for 20 d before extraction at 20 and 100 °C in 1% SDS. The 60–70S complex seems to be intact, but, on melting, the complex liberates only small fragments of RNA of approximately 4S (Fig. 3h). This suggests that the virus preparations have an associated ribonuclease capable of producing breaks in the 35S strand while they are complexed in the 60–70S form. Whether the large amount of 4S RNA detected in all HEV preparations so far examined (Fig. 3e–f) represents degraded viral RNA or host tRNA associated with the virions is not known.

Our inability to detect protein in the 60–70S RNA complex from TGEV does not exclude the possibility that there is a very small amount that is dissociating from the RNA at elevated temperatures in the presence of SDS. The similarity of behaviour and size between the coronaviral RNA and RSV RNA, together with the liberation of 4S RNA on melting suggests strongly that the TGEV and HEV 60–70S complex is held together by RNA–RNA interactions as is the RNA from oncornaviruses. We hope to characterise further the 60–70S complex and determine whether the viral 4S component is in fact host tRNA. Although the replication of these two groups of viruses is fundamentally different, the coronaviruses being entirely cytoplasmic in contrast to the essential nuclear phase of the oncornaviruses, a similarity in the structure of the genomes of the two groups raises interesting implications for the phylogeny of the RNA tumour viruses.

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Presence of factor VIII-related antigen in blood platelets of patients with Von Willebrand's disease

VON WILLEBRAND'S disease (VWD) is an autosomally inherited disorder characterised by low factor VIII activity (antihaemophilic factor, AHF), prolonged bleeding time, reduced retention of platelets in a glass bead column and abnormal distocetin-induced platelet aggregation. The prolonged bleeding time in VWD has been attributed to the absence of a plasma factor, the von Willebrand factor (VWF), as shown by a correction of the bleeding time after infusion of normal and haemophilic plasmas¹. Addition of purified factor VIII *in vitro* specifically corrects the abnormal platelet retention and ristocetin aggregation in VWD^{2–5}, whereas transfusion of similar material into dogs with VWD also corrects the prolonged bleeding time (B.N.B., W. J. Dodds, J. A. van Mourik, J.J.S. and

W. P. Webster, unpublished). This led to the suggestion that factor VIII is closely related if not identical to VWF, although dissociation of factor VIII procoagulant activity from factor VIII-related antigen (F VIII-RA) is observed in certain conditions^{6,7}. The plasma concentration of F VIII-RA is usually reduced in VWD suggesting a reduced synthesis of factor VIII (VWF)⁸. In contrast to this we now report the presence of normal concentrations of F VIII-RA in platelets of patients with VWD. This F VIII-RA supported aggregation induced by ristocetin in a washed platelet system⁹, a property of factor VIII which has been attributed to VWF activity.

Human blood platelets were washed according to the method of Karparkin¹⁰. ACD-blood was centrifuged (10 min, 200g, 20 °C). Platelet rich plasma was diluted in 5 volumes of Krebs-Ringer buffer (pH 7.4) containing 9 mM Na₂-EDTA. After centrifugation (15 min, 1,000g, 4 °C), the platelets were washed once in Krebs-Ringer buffer containing 9 mM Na₂-EDTA and twice in Krebs-Ringer buffer containing 1 mM Na₂-EDTA. The platelet pellet was finally resuspended in Krebs-Ringer buffer containing 30 mM glucose and 1 mM ε-amino caproic acid. In the final washing fluid the protein content was lower than 50 μg ml⁻¹ (ref. 11), and the concentration of F VIII-RA, measured by electroimmunodiffusion was below 0.05 U ml⁻¹ (ref. 12). 1 U F VIII-RA was defined as the amount present in 1 ml pooled normal plasma prepared from 40 healthy subjects¹². In the final suspension (1 × 10⁶–3 × 10⁶ platelets μl⁻¹) the platelets were disrupted by freezing and thawing (four times) followed by centrifugation (60 min, 30,000g, 4 °C).

Ten suspensions of normal human platelets were tested. The concentration of F VIII-RA detected in the supernatant was 0.15 U mg⁻¹ platelet protein (range 0.11–0.25) or 43 U per 10¹¹ platelets (range 19–94). Expressed per platelet volume (8 × 10⁻¹⁵ l) (ref. 13) the concentration of F VIII-RA was 60 times (range 21–156) higher than that in plasma. Similar values were reported by Nachman and Jaffe¹⁴.

Platelet F VIII-RA showed a reaction of identity with plasma F VIII-RA, when tested in immunodiffusion using a rabbit anti-factor VIII serum and the antisera raised against the low ionic strength components (ref. 15 and B.N.B., J. van Mourik, S. de G., J.M.H.-H. and J.J.S., unpublished). Cross-immunoelectrophoretic analysis revealed an electrophoretic mobility comparable with that of plasma F VIII-RA.

Howard *et al.*¹⁶ quantitated F VIII-RA on intact washed normal platelets and concluded that F VIII-RA was firmly bound to the membrane fraction. A similar conclusion was reached by Bloom¹⁷ using an immunofluorescent technique. On the other hand, the localisation of F VIII-RA throughout the megakaryocytic cytoplasm is in agreement with its localisation inside the platelet¹⁸. Nachman and Jaffe¹⁴ reported the presence of F VIII-RA in subcellular membrane and granula fractions. We examined this localisation by a previously described indirect immunofluorescent technique^{19,20}. Suspensions of intact washed platelets were incubated with antifactor VIII in suspension, washed again, and incubated with fluorescein isothiocyanate labelled horse anti-rabbit globulin (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam). A drop of this suspension was studied under the fluorescence microscope. Most platelets were unstained, whereas a vivid granular staining was obtained after disruption of membranes by air drying of a drop of the same platelet suspension on a glass slide. The specificity tests have been described in detail elsewhere²⁰. Staining with normal rabbit serum instead of with antifactor VIII was used as a control. The difference between staining in suspension or staining after air drying suggests that F VIII-RA is present inside the platelets.

In agreement with the results of Howard¹⁶ and Nachman and Jaffe¹⁴ no factor VIII activity could be detected in

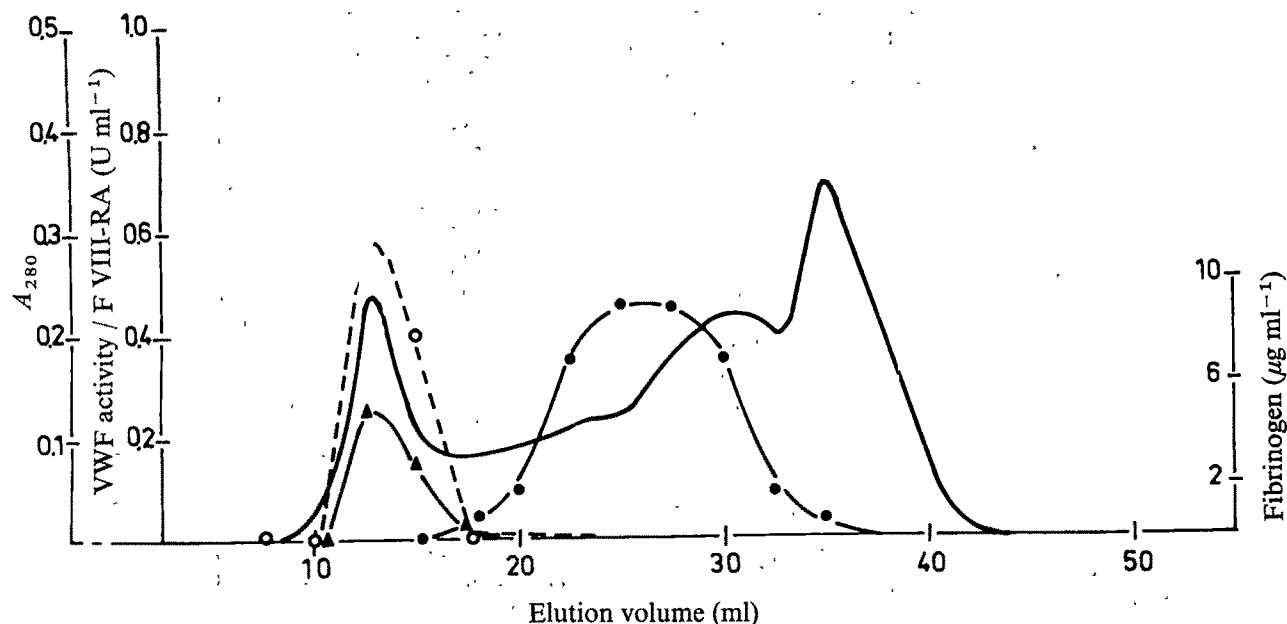


Fig. 1 Chromatography of a platelet lysate obtained from a patient with VWD. A column (K15/30, Pharmacia, Uppsala) was packed to a gel height of 24 cm with Sepharose 6B (Pharmacia, Uppsala) and equilibrated with a barbitol-saline buffer (0.0167 M barbitol, 0.125 M NaCl, pH 7.0) containing 3.3% dextran (Rheomacrodex, molecular weight 43,900, Pharmacia AB) and 1 mM ϵ -amino caproic acid. Platelet lysate obtained from 70 ml of normal human blood or blood from patients with VWD in a final volume of 1–2 ml was applied to the top of the column and the column was eluted with buffer at 15 ml h⁻¹. All procedures were performed at 4 °C. Fractions were analysed for: (1) absorbance at 280 nm (—○—); (2) F VIII-RA as measured by Laurell technique (○)¹²; (3) fibrinogen as measured by Laurell technique (●)²⁷; and (4) presence of VWF activity as determined by support of ristocetin aggregation in washed platelet suspensions (▲—▲)⁹.

the supernatant of platelet lysates. It should be noted, however, that a clot-promoting activity was detected using a one stage factor VIII assay²¹. This clot-promoting activity could not be inhibited by human circulating factor VIII inhibitors, and was also detected in the supernatant of lysed haemophilic platelets. The clot promoting activity was therefore not attributed to factor VIII and probably resulted from tissue factor activity released by contaminating leukocytes. The lack of factor VIII activity could not be attributed to the freezing and thawing procedure, because factor VIII activity was already absent after the first freeze-thaw manipulation. The presence of proteolytic enzymes, as a cause of the decreased factor VIII activity is more difficult to exclude. However, addition of 1 mM ϵ -amino caproic acid or other proteolytic inhibitors (benzamidine, 1 mM; Soya bean trypsin inhibitor, 0.02 mg ml⁻¹; and phenyl methyl sulphanyl fluoride, 1 mM) to the final washing fluid before disruption of the platelets had no effect on the measurement of factor VIII activity. Moreover the supernatant obtained after freezing and thawing did not inactivate the clot promoting activity of purified factor VIII during 24 h of incubation at 37 °C. This indicates that the factor VIII identified in platelets as F VIII-RA is biologically inactive. Similar observations were made for human endothelial cells, FVIII-RA was present¹⁸ but devoid of factor VIII activity²².

The presence of F VIII-RA has been demonstrated with haemostatic plugs²⁰. Electron microscopic observations of haemostatic plugs in rabbits and dogs have shown that the platelets are mostly degranulated²³. In preliminary investigations we found that collagen as well as thrombin (final concentration 5 U ml⁻¹, Roche, Basle) produced little or no release of F VIII-RA from intact platelets which explains the presence of F VIII-RA in haemostatic plugs that had undergone release.

Normal concentrations of F VIII-RA were detected in platelets from patients with haemophilia A. F VIII-RA was also measured in platelets from 15 patients with VWD (Table 1). All patients except No. 10, who was considered to manifest VWD type II (ref. 24), had low or absent

F VIII-RA in their plasma and fulfilled the criteria of VWD. In two patients (Nos 4 and 11) no F VIII-RA was detected in the platelets, whereas all other patients had normal concentrations of F VIII-RA in their platelets. These results were supported with an indirect immunofluorescence technique¹⁹ applied to drops of platelet suspensions air-dried on a glass slide. A positive result was obtained with all patients, except one (No. 4) of the two patients without F VIII-RA in their platelets. The other patient was not tested. It is of interest that the patient (No. 11) without F VIII-RA in his platelets is a brother of patient No. 15, suggesting that the absence of F VIII-RA in platelets of patients with VWD is not hereditary.

F VIII-RA from normal human platelets was purified by gel chromatography according to van Mourik and Mochtar²⁶ (Fig. 1). Both F VIII-RA and VWF-activity were detected in the void volume (V₀) fractions from these columns, which is in agreement with observations for plasma factor VIII². Figure 1 shows the results for the chromatogram lysates of VWD platelets. In 3 experiments, the V₀ fractions contained both F VIII-RA and VWF-activity, whereas no VWF-activity and F VIII-RA was detected in the V₀ of VWD platelet lysates containing no F VIII-RA.

These results indicate that although F VIII-RA is reduced or absent in the plasma of VWD patients, F VIII-RA can be detected in the platelets of most of these patients.

Two reports in the literature are in conflict with our results. Howard *et al.*¹⁶ failed to demonstrate any F VIII-RA in the platelets of one patient with severe VWD and Coller *et al.*²⁸ found platelets from two patients to be negative in immunofluorescence studies. Shearn *et al.*²⁹, however, comparing five different heterologous antifactor VIII antisera found that the platelets from one patient with severe VWD were positive with four of the five antisera using an immunofluorescence technique. The presence of the antigen was also demonstrated by cross immunoelectrophoresis. It should be noted that these studies were performed with a very limited number of patients, whereas the techniques used also differ from ours. One explanation for the discrepant results, may be contamination of the antifactor VIII sera

Table 1 Platelet factor VIII-related antigen (F VIII-RA) in VWD

Patient	FVIII-RA/mg platelet protein (U)	FVIII-RA/10 ¹¹ platelets (U)	FVIII activity plasma (U ml ⁻¹)	FVIII-RA plasma (U ml ⁻¹)	Bleeding time ²⁵ (min)
1	0.18	35	0.14	< 0.05	7
2	0.14	56	0.23	< 0.05	9
3	0.04	16	0.13	< 0.05	7
4	< 0.01	< 0.01	0.12	< 0.05	> 15
5	0.15	35	0.11	< 0.05	—
6	0.27	60	0.05	< 0.05	> 15
7	0.18	52	0.15	< 0.05	> 15
8	0.26	67	0.12	< 0.05	10
9	0.23	90	0.18	< 0.05	—
10	0.24	47	0.44	0.80	> 15
11	< 0.01	< 0.01	0.15	0.10	—
12	0.08	14	0.10	0.10	—
13	0.14	38	0.11	0.05	—
14	0.36	120	0.35	0.40	9
15	0.16	34	0.21	0.25	6

with antibodies produced in rabbits against platelet fragments, which may be carried along in the purification of factor VIII. The following observations suggest that in our experiments the antigen detected by rabbit anti-factor VIII in platelets is indeed F VIII-RA.

(1) The platelet antigen could not be distinguished from plasma factor VIII when tested in immunodiffusion and cross immunoelectrophoresis, using antifactor VIII and the antisera against the low ionic strength components of factor VIII. (2) The positive immunofluorescence of haemostatic plugs was completely blocked by absorption of antifactor VIII with purified factor VIII, the characteristics of which have been described in detail^{15,26}. (3) In preliminary experiments antibodies were prepared against normal and VWD platelet F VIII-RA. These antisera precipitated with purified factor VIII when tested in immunodiffusion experiments.

Previously it has been suggested that the capacity to synthesise F VIII-RA is reduced in VWD. This was concluded from the fact that F VIII-RA is reduced or absent in plasma of patients with VWD. Our findings, however, suggest that the platelets, or more likely the megakaryocytes retain the capacity to synthesise F VIII-RA. Another possible explanation for the presence of F VIII-RA in VWD platelets is that the platelets selectively sequester F VIII-RA from an extracellular source such as plasma or endothelial cells. The fact that F VIII-RA from normal as well as from VWD platelets supports ristocetin-induced aggregation may indicate that platelet F VIII-RA is also identical to VWF. VWF has been deemed necessary for normal haemostasis because patients with VWD who lack this factor have a prolonged bleeding time. The presence of F VIII-RA in haemostatic plugs²⁰, and blood platelets suggests that it serves some useful function in haemostasis. A direct haemostatic role of platelet F VIII-RA seems unlikely because it is not released from intact platelets and is present in platelets of patients with VWD who have prolonged bleeding times. Although plasma F VIII-RA seems to be more important in haemostatic plug formation the presence and synthesis of F VIII-RA in endothelial cells and its localisation in blood platelets suggest additional haemostatic functions for this molecule.

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Corrigendum

In the article "The origin of nuclei and of eukaryotic cells" by T. Cavalier-Smith (*Nature*, **256**, 463; 1975) the two unlabelled arrows at the top right of Fig. 5 should read (from the top) Prasinophyta and Charophyta, respectively.

Erratum

In the article by B. Donzel and M. Goodman (*Nature*, **256**, 750; 1975) the title should have read "Synthesis and conformations of hypothalamic hormone releasing factors: two TRF analogues containing backbone N-methyl groups" and not as printed.

matters arising

Strainmeter technology

WE agree with Sydenham¹ that many problems remain to be investigated with strainmeters and that many results obtained so far are ambiguous or contradictory. Measurements of strain (especially long period strain) are beset by a host of problems not found in such areas as magnetometry, so it may, therefore, be unreasonable to expect strainmeters to be as simple as magnetometers. Further, it may be time to opt for a smaller number of higher performance instruments and to reconsider the continued deployment of more and more strainmeters in a given area.

Although the measurement of strain is in principle differential, in practice the long term nature of most strain measurements requires great stability in the reference length, the realisation of which is tantamount to constructing a length standard.

Conventional materials, such as fused silica, have coefficients of expansion of about $10^{-6} \text{ }^{\circ}\text{C}^{-1}$ so that thermal fluctuations must be held to less than $10^{-4} \text{ }^{\circ}\text{C}$ over the entire length of the instrument if the thermally induced strain signal is to be kept below 10^{-10} (in order to measure a particular component of the Earth tides to an accuracy of 1%, for example).

Sydenham has shown³ that this can be done for very short strainmeters (10 m) in the laboratory. The ancillary apparatus required to achieve microdegree stabilities is, however, far from simple, even for 10-m instruments, and difficulties in stabilising longer instruments can be expected to increase more than linearly with length.

The length of fused silica strainmeters also depends on the ambient humidity in a nonlinear manner (F. Homuth, unpublished thesis). Thus, stabilisation of the humidity is also required. That could be accomplished, at least to the first order, by enclosing the instrument in an evacuated pipe, in which case, of course, it would look exactly like a laser strainmeter.

By way of contrast, saturated absorption stabilisers routinely achieve stabilities and reproducibilities of 10^{-11} or better without any sort of thermal or environmental isolation. These data on stability and reproducibility are obtained by direct comparison between two independently stabilised devices.

It is true that the interferometer comprising the heart of the laser strainmeter is sensitive to fluctuations in atmospheric pressure and temperature, the fractional

change in the optical path length being 2×10^{-10} per mtorr. A simple mechanical pump can easily evacuate the interferometer to a few mtorr and can maintain the pressure at that level indefinitely. The temperature dependence of the index of refraction at a few mtorr is negligible.

Until the advent of saturated absorption stabilisers, attempts to measure long term strain accumulations with conventional strainmeters were severely limited by the signals produced by various environmental effects. There is no question that conventional strainmeters can be used for conventional seismology and even for normal mode work (periods of less than about 1 h). In fact, many of the best normal mode data come from quartz rod strainmeters⁴. But for periods of longer than 1 h it becomes more and more difficult to isolate adequately the reference length.

Thus, our conclusions are quite different from those of Sydenham. We feel that there is no 'best' overall strainmeter, and that each instrument has advantages and shortcomings.

Laser strainmeters are usually faulted for their high cost and low reliability. But neither the cost nor the sophistication of a laser instrument increases very much with length, so that long instruments are quite a bit cheaper per metre than are short ones. We suggest that the opposite may be true for instruments requiring good thermal stability over their entire length. If, as Homuth (unpublished) suggests, stabilisation of the ambient humidity turns out also to be necessary, the cost of the total system will be even higher, and the reliability, presumably, lower.

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SYDENHAM¹ has failed to appreciate that problems exist in studies of Earth strain which will not be solved by producing the 'ultimate' strainmeter. Strain signals cover a wide frequency range, and signal sources and noise vary across the spectrum. The correct way to assess instrument performance is to examine noise levels as a function of frequency (see ref. 2, for example). An early invar wire strainmeter

designed and constructed by Sydenham³ achieved signal-to-noise ratios of up to 100 on tidal peaks and although the instrument had defects, a poor signal-to-noise ratio was not the most important. As Sydenham has never published any power spectra it is not possible for others to compare the behaviour of their instruments with his. His interest in drift and stability amounts to an avid concern with only the lowest frequencies of the spectrum. Using drift as a criterion for comparison, however, it is interesting to note that Sydenham's early instrument has exhibited less long term drift than he now claims for quartz instruments⁴ although even that level is too high to permit a strainmeter to compare in the long term (more than 1 yr?) with geodetic techniques for determining secular strain.

Sydenham's discussion of tensor arrays is also misleading. Given six instruments and a good site, a tensor array will not usually give maximum useful information. Geophysically important results (different examples are given in refs 5–9) can be obtained using single instruments or linear arrays of horizontal strainmeters and these also reveal site effects^{7,10,11}. Should a site prove to be badly chosen perhaps it is best to go elsewhere.

No published data suggest that mechanical strainmeters are universally better than optical strainmeters (or vice versa). We register surprise that since Sydenham favours borehole instruments he makes no comment on the borehole dilatometer developed at the Carnegie Institution, Washington¹² or the 'Geotech' borehole strainmeter¹³.

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SYDENHAM REPLIES—My paper was compiled in late 1973, and represents events of that time. A vital word—'headed'—did not appear at the end of the title as intended.

By early 1974, experimental Earth-strain research had reached a stage at which mechanical instrumentation was no longer a significant unknown of any programme. The next stage in maturity was to know how to obtain geophysical contributions with the hardware. In 1974, the modifying effects of cavities and regional topography were universally recognised and serious studies were initiated (earlier work with linear arrays was simplistic and often non-productive). Important and unexpected results have emerged from this approach (ref. 1 and D. P. Blair, unpublished thesis) showing how and to what extent expected strain magnitude is altered. Blair (unpublished), extending Harrison's¹ finite-element work from two into three dimensions has found that topography selectively alters the amplitudes of the tidal spectral components. This result apparently explains that topography was the influencing parameter sought without success at the Queensbury site²; yet King questions my earlier predictions of the relevance of a tensor study.

Reference about strain-creep using linear arrays was indeed omitted as it is more usually studied using quite coarse instruments (though in my original manuscript it was suggested that creep theory, as studied by materials scientists, could be relevant to an understanding of creep mechanisms). This concept has since been invoked³.

I suggested that theory might help reduce the number of components needed to understand how strains relate at different places. Available techniques now allow the prediction of site effects using only a geological map, a topographical map and computing ability. We can now eliminate most known causes of incompatibility between theory and practice, leaving the interesting differences to be investigated. Deep-sea cotidal charts, for instance, might soon be updated using corrected Earth-strain data (D. P. Blair, unpublished thesis). Resolution of the interval argument could be near: structural studies based on topographical studies should give quantitative guidelines.

My work on drift reduction in instruments led to the development of the mechanical instruments King's group now uses. A very real advantage of high stability is the ease it offers of transducer design and recording. Quartz chain catenary instruments need no autoranging recorder nor any adjustments over yearly periods. Long-period differential drift

between two different quartz instruments is no greater than 5 parts in 10^{12} per hour⁴. Very long baseline interferometers excepted, no available geodetic technique can provide drift as low.

Frequency spectra could provide a means of assessing instrument performance but only if the power contributions of the Earth and the instrument can be distinguished separately. I have seen no point in publishing the tidal spectra for they contribute little. The work of Berger and Levine⁵ referred to by King represents excellent spectral analysis, but does it provide useful knowledge? If a site topography alters the amplitude¹ and composition of signal (ref. 1 and D. P. Blair, unpublished thesis) it does likewise to the tensor fluctuation, noise. Comparisons could be out by a factor of 0-3 if site coefficients differ greatly.

Is not the ultimate strainmeter one that provides adequate quality data, ease of recording, ability to be used anywhere in any direction, is the cheapest, the quickest to install, creates the smallest cavity error, automatically compensates for any local thermoelastic effect, and gives high reliability? A core-hole instrument (D. Curtis and P. H. S., unpublished) offers that at the economic ratio of 150 to one (compared with the first kilometre-length surface laser instrument). Until laser instruments reveal significant geophysical information not gained by well designed, mechanical equivalent extensometers, I cannot see how it can be argued that they are worthwhile geophysical tools. Surely, Levine does not justify laser expenditure on cost per unit length at this stage of uncertainty about the ideal interval?

Levine's comments on environmental effects on quartz can be misleading. Ambient control in subsurface installations is usually just within needs. Even if quartz is unstable with humidity change, it is easy to control relative humidity with a passive flexible enclosure.

The cost of advanced total environmental control⁶ is no more than that of a supplying and running a fail-safe evacuated enclosure. I again stress the point, however, that this line of reasoning is not fundamentally correct. An instrument having the same thermal coefficient (made of stainless steel, not quartz) as the host rock in which it is buried, requires no ambient compensation.

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¹ Harrison, J. C., *Trans. Am. geophys. Un.*, 56, 1151 (1974).

² Bilham, R. G., King, G. C. P., and McKenzie, D. P., *Geophys. J. R. astr. Soc.*, 37, 217-226 (1974).

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Extinction of taxa and Van Valen's law

VAN VALEN¹ has proposed an evolutionary-law that has attracted considerable attention²⁻⁶. He showed that plots of survivorship of taxa as a function of age are commonly log-linear (see Fig. 1 of ref. 1). This he interpreted to mean that the probability of extinction of a taxon is independent of its age, and thus formulated the law: "The effective environment of the members of any homogeneous group of organisms deteriorates at a stochastically constant rate."¹ We show here that survivorship analysis of the log-linear curves constructed by Van Valen does not warrant the interpretation that he assigned to it; support for Van Valen's law must be sought elsewhere.

In life table analysis one of two methods are used to construct a survivorship curve⁷⁻⁹. Horizontal, dynamic, or cohort life tables require a set of optimisms of even age (usually taken at birth), whose survivorship curve is derived from a complete analysis of the timing of deaths. All that is required is to record the age of death of each member and plot the number of survivors for each age class. Vertical, static, or actuarial life tables differ in that a sample of living organisms (or of ageable remains of dead organisms) is collected over a series of ages¹⁰. The critical assumption of the vertical life table is that the proportion of organisms within each age class decreases as a function of the age-specific death rate.

Van Valen claimed that his data were compiled in cohort-type tables, but Raup⁵ has argued that Van Valen used vertical life tables for extinct taxa. In fact, Van Valen combined the two types of life table analysis for both living and extinct taxa. The abscissa of his plots for extinct taxa is time of survival, arranged in such a way that all taxa are scaled as if they had originated simultaneously. For living taxa his abscissa scaling procedure is a straightforward reversal of the procedure for extinct taxa, since all living taxa are contemporary but evolved at various times in the past. In both cases the data seem to form a cohort, but they clearly do not. In the case of extinct taxa the scaling procedure is entirely responsible for the appearance of a cohort, but since the actual times of origin of all points are not the same, there can be no cohort analogous to an ecological life table. In reality the data constitute a vertical life table plotted to appear as a cohort, giving a survivorship curve without additional calculations. In the case of living taxa the data form a reversed cohort table, enabling survivorship to be estimated

directly, assuming that such a reversed table is meaningful.

Van Valen's graphical analysis does not contain the usual interpretation of survivorship probabilities. Ordinary survivorship analysis is specific for a particular sample of organisms over a short time interval and a reasonably constant environment. Thus, analysis of survivorship and mortality reveals patterns of life history in relation to some set of environmental (biotic and abiotic) interactions. This is why log-linear survivorship curves of any kind of organisms are rare, since log-linear curves imply that all members have an equal chance of dying regardless of age. Van Valen's plots are fundamentally different. The time scale is in tens of millions of years, but environmental conditions have not been anything near constant over such long time spans¹¹. Furthermore, members of the 'population' (the set of data points) do not necessarily share many common characteristics. They are hardly a homogeneous group reacting to a set of environmental conditions in the same way; they do not necessarily coexist or even share similar environments. Van Valen's survivorship curves aggregate greatly both the time-age scale and environmental conditions.

Given the scaling used by Van Valen, log-linearity is to be expected regardless of whether the probability of extinction of a taxon is or is not independent of age. Four levels of aggregation contribute to the appearance of log-linearity. (1) The environment-taxa relationship is lost by using taxa that have existed in different places at different times and aggregating them into the analogy of a population; environmental variations over time and space are effectively eliminated. (2) The long time scale ensures that nonlinearity could result only from extraordinary irregularities in extinction rates lasting for epochs. Even major waves of extinctions such as occurred at the Permian-Triassic and Cretaceous-Tertiary boundaries are reduced to small irregularities in the curves. (3) Elimination of points from both ends of the curves (representing taxa of relative short existences and those surviving for very long periods) reinforces linearity. Raup⁵ has already made this argument effectively. (4) The use of high order taxa rather than lower level taxa (ideally, species) makes the data points insensitive to variation among taxa and among the environments.

Because of their high level of aggregation, Van Valen's curves effectively eliminate all but the most extraordinary events, that is, they are relatively insensitive to perturbation. It is the aggregated scale itself which is responsible for log-linearity. The inclusion of

stochastic events to explain variation then requires a very broad definition of 'stochastic'.

At least three different questions must be distinguished in discussions about extinction probabilities. One question is whether the probability of survival of a taxon or group of taxa is the same at all geological times. This question must be answered in the negative—the fossil record shows that during periods such as the Permian-Triassic boundary any existing taxon had, on the average, a greater probability of becoming extinct than at other geological time. A second question is whether, at any given geological time, the probability of extinction is the same for all taxa of a given group. The answer to this question is likely to be also in the negative—at any given time, different related taxa may have different probabilities of becoming extinct. The third question is whether at any given geological time, the probability of extinction for any one taxon is independent of its age. Evolutionary theory predicts an affirmative answer to this third question. Natural selection promotes adaptation to the current environments of a species but does not anticipate the future. Confirmation of this prediction must await further studies.

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² Raup, D. M., Gould, S. J., Schopf, T. J. M., and Simberloff, D. S., *J. Geol.*, 81, 525-542 (1973).

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⁴ Van Valen, L., *Nature*, 252, 298-300 (1974).

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VAN VALEN REPLIES—Foin, Valentine and Ayala¹ have produced a curious mixture of misinterpretation and conceptual confusion. I was careful² to avoid saying that 'the probability of extinction of a taxon is independent of its age'. This is true only for the mean probability (propensity) for a set of taxa, and I gave reasons³ for believing that individual taxa do often have different probabilities at different ages.

The reasons derive from both evidence and conventional theory, which therefore does not predict constancy as Foin *et al.* claim. Some subtaxa, for example those that are widespread and that contain many species, do have a low probability of extinction, as expected. Together with the observed mean constancy, this requires that the distribution of probabilities of extinction itself be regulated over time², so that the mean probability for older taxa is the same as that for younger ones. Some taxa therefore become more susceptible with age.

The critics seem not to have heard of composite life tables, which are a standard type³⁻⁵. In this type the data are asynchronous and must usually be analysed by the dynamic method. Simpson⁶ first made composite life tables of taxa from the fossil record. Raup⁷ did not, as claimed, make the elementary mistake of believing I treated extinct taxa by vertical analysis, although I disagree with part of what he did say.

One of the few advantages in using the fossil record for biological analysis is its averaging effect⁸. Local spatio-temporal peculiarities are indeed averaged out. This enables general properties to reveal themselves while 'noise' is minimised.

Any realistic combination of taxa with different characteristic probabilities of extinction gives a concave curve (convex in the terminology of analysis). The fact that the curves are rarely concave (and then only for groups with other evidence of heterogeneity, for which the same subgroups are found as for survivorship analysis) implies that the mean probability is roughly constant over long intervals. There are many causes of the various small irregularities in the curves (ref. 2 and L.V.V., unpublished). Low stratigraphic resolution makes the upper left extreme of the curves unreliable, and the low numbers of taxa at the bottom of a log scale make exceptions at the lower right extreme, even when real, of little importance. Appreciable nonlinearity is nevertheless easily detectable: I have in fact detected ostensible exceptions in both directions⁹, which do not affect the interpretation of a long term constancy.

Foin *et al.* have a somewhat subtle confusion reminiscent of the denial¹⁰ of a long term constancy in protein evolutionary rates. In neither case will "capricious" factors¹⁰, even acting over a long time, give the observed similarity in mean rate for different long intervals. They give a quasi-random walk of rates that is unconstrained by any predictable controlling distribution and so diverges wildly. Even an ordinary Markovian random walk usually diverges and so would usually

give a nonlinear curve. The observed degree of similarity can occur only by the existence of feedback control on a single mean value that is stable over long intervals¹¹ in spite of 'random' perturbations, and it is the very existence of this regulated mean which is the most interesting aspect of both kinds of phenomena.

The claim that I didn't use "lower level taxa (ideally, species)" is strange. I used all available and adequate data: 5 graphs were for species, 34 for genera, and 17 for families. The evidence of constancy is similar for each category. Because supraspecific taxa have different numbers of species, their observed linearity is evidence² for the ecological reality of supraspecific taxa.

I gave the first demonstration² that the mean probability of extinction (and that of branching) of subtaxa of a higher taxon varies in geological time. All previous studies had confounded either the absolute number of subtaxa, or the length of geological periods, with this probability. I also gave evidence that there is real heterogeneity in the probability of extinction of contemporaneous subtaxa.

Here is an apparent paradox: individual subtaxa vary both contemporaneously and over time in their susceptibility to extinction, and the mean susceptibility varies over time, yet the mean is constant over long intervals. The Red Queen's Hypothesis^{2,11,12} attempts to resolve the paradox by treating evolution as mostly ecologically rather than genically controlled, and as a zero-sum game of a kind which game theory has ignored. (In response to an earlier discussion¹³ what else is needed for the resolution is simply an independent maintenance of taxonomic diversity, which is an empirical fact whatever its detailed explanation.) As a by-product this ecologically selectionist theory explains the major features of molecular evolution at least as well as the genically neutralist and genically selectionist theories. Some of these congruences have been published¹¹, and I have found that one of the two apparent difficulties¹¹ of the hypothesis does not exist when explicitly tested¹⁴.

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Sodium flux, action potential and temperature dependence

LANDOWNE¹ and Cohen and Landowne² reported that extra Na flux during the action potential in squid axons increased little with decreasing temperature, the overall experimental Q_{10} being about 1/1.4. Hodgkin and Huxley³, however, computed a Q_{10} of 1/2.87 by assuming that temperature affected only the rate constants governing ion permeability changes. This discrepancy between experiment and theory was interpreted^{1,2} as indicating that nerve membranes do not function by changes in ion permeability⁴. We have used Hodgkin-Huxley-type equations³ and incorporated experimental temperature dependencies of the maximum possible Na and K conductances (\bar{g}_{Na} and \bar{g}_K) and the nonspecific leak conductance (g_L). Our results seem largely to resolve the discrepancy. A similar resolution of discrepancies between measured and computed K fluxes was proposed by FitzHugh and Cole⁵.

Membrane action potentials were computed with Hodgkin-Huxley equations, modified for *Myxicola*⁶, using a modified fourth-order Runge-Kutta algorithm, and the six-parameter model⁶ (without reference to inactivation parameters determined with conditioning pulses). Q_{10} s of the parameters, from Schauf's results with *Myxicola*⁷, are: 2.64 for Na activation rate constants (α_m and β_m), 2.56 for Na inactivation rate constants (α_h and β_h), 3.02 for K activation rate constants (α_n and β_n), 1.37 for \bar{g}_{Na} , 1.50 for \bar{g}_K , and 1.34 for g_L . We used these equations because good temperature data were available. We regard them only as an empirical description of the action potential.

Figure 1a and b shows action potentials and the time courses of the Na currents, computed at 5 °C and 15 °C. Table 1 shows temperature effects on time to rise, time to decline, and duration at +50 mV of the action potential. The Q_{10} of the duration at +50 mV (1/2.75) agrees well with the experimental value⁷ of $1/2.47 \pm 0.2$. Also shown are values for net Na movement during the action potential, (pmol cm⁻²) obtained by integrating the Na current curves of Fig. 1a. Taking the Q_{10} of the net Na entry as a measure of the Q_{10} of the extra Na flux, we get 1/1.82 which agrees reasonably well with the experimental value (1/1.4), considering that the preparations differed

and we simulated single membrane action potentials, not repetitive propagated responses. Computations made excluding the temperature dependency of \bar{g}_{Na} , \bar{g}_K and g_L gave a Q_{10} of 1/2.44, much as Hodgkin and Huxley³ reported and divergent from the experiments.

We find, therefore, no serious discrepancy between the measured temperature dependency of the extra Na flux during an action potential and that computed

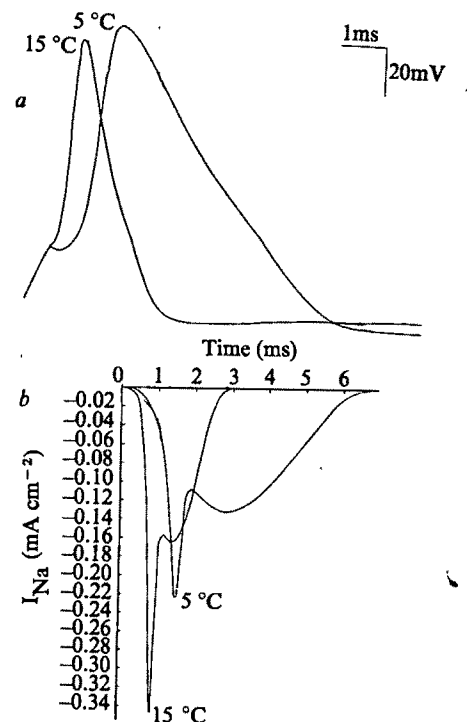


Fig. 1 a, Two computed *Myxicola* action potentials, at the temperatures indicated. Computations have been made according to the six-parameter model of Goldman and Schauf⁶. The experimental temperature dependencies of \bar{g}_{Na} , \bar{g}_K and g_L , as well as those of the rate constants governing the ion permeability changes have been included. b, Computed time course of the Na currents for the two computed action potentials (a)

from Hodgkin-Huxley kinetics and so major conclusions on the mechanism of ion translocation do not seem warranted. This agrees with reports of a close correspondence between net Na flux determined from voltage clamped currents and

Table 1 Computed temperature effects on the action potential in *Myxicola*

	Time from +25 mV to peak (ms)	Time from peak to 0 mV (ms)	Duration at +50 mV (ms)	Net Na movement (pmol cm ⁻²)
5 °C	0.85	4.38	2.75	5.13
15 °C	0.44	1.73	1.00	2.82
Q_{10}	1/1.93	1/2.54	1/2.75	1/1.82

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that determined from tracer flux⁸⁻¹⁰, a method which does not use a kinetic model.

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² Cohen, L. B., and Landowne, D., *J. Physiol., Lond.*, **236**, 95-111 (1974).

³ Hodgkin, A. L., and Huxley, A. F., *J. Physiol., Lond.*, **117**, 500-544 (1952).

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¹⁰ Mullins, L. J., Adelman, W. J., and Sjodin, R. A., *Biophys. J.*, **2**, 257-274 (1962).

LANDOWNE AND COHEN REPLY—Goldman *et al.*¹ state that they find no serious discrepancy between the measured temperature dependence of the extra sodium flux during an action potential and that computed from Hodgkin-Huxley kinetics.

We have discussed² this discrepancy, or the lack thereof, in a qualitative fashion and both we² and Fitzhugh and Cole³ concluded that there is still a discrepancy even when the effect of temperature on the maximum conductances was included. Although Hodgkin and Huxley succeeded in predicting the electrical behaviour of the axon membrane based on their measurements of currents resulting from potential steps, they noted that at room temperature there was disagreement between the computed ion fluxes and those found experimentally⁴. We have also computed the fluxes predicted by the Hodgkin-Huxley equations and find there is considerable disagreement with experimental data at many temperatures.

The Hodgkin-Huxley equations were solved every three degrees from 3 to 27 °C and the calculated extra unidirectional tracer fluxes for membrane action potentials are shown as the solid curves in Fig. 1, together with the experimental data. Curve 1 represents the fluxes predicted by the unmodified Hodgkin-Huxley equations with a Q_{10} of 3.0 on the rate constants and 1.0 on the conductances. These calculated fluxes are within 1% of those given in Table 5 of Hodgkin and Huxley's original calculations at 6.3 and 18.5 °C (ref. 4). Curve 2 shows the effect of including a Q_{10} of 1.5 on the conductances and curve 3 was generated by using the Q_{10} s given by Goldman *et al.*¹. By taking the ratio of the net fluxes at 5 and 15 °C from the calculations for curve 3, a Q_{10} of 1.8 is obtained, similar to that of Goldman *et al.*¹. Clearly, none

of the curves fits the experimental data very well although, as suggested previously^{2,3} and by Goldman *et al.*¹, the fit is improved by including the effect of temperature on the maximum conductances. All the computed action potentials look qualitatively similar to experimental action potentials. Thus it seems that the appearance of the computed action potentials is not a good criterion for prediction of the appropriate fluxes.

The experimentally observed Q_{10} s were 1/1.4, 1/0.7 and 1/1.6 for the extra Na influx and 1/1.0, 1/1.3 and 1/1.2 for the extra Na efflux, for a variety of experimental conditions. We think that it is quite possible that these numbers are smaller than 1/1.8.

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¹ Goldman, L., Hahn, R., and Beginisich, T., *Nature*, **257**, 516-517 (1975).

² Cohen, L. B., and Landowne, D., *J. Physiol., Lond.*, **236**, 95-111 (1974).

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⁴ Hodgkin, A. L., and Huxley, A. F., *J. Physiol., Lond.*, **117**, 500-544 (1952).

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Equivalence principle

BOTH Bishop and Landsberg¹ and Kilminster in his review² use the argument

Gravitational redshift

→ Retardation of clocks

→ Space-time curvature

where we have identified the "non-Minkowskian metric"¹ and the phrase "the geometry of space-time is modified"² with space-time curvature. We have shown³ this argument (which is due to Schild^{4,5}) to be invalid and gave the rather obvious counterexample of a uniform gravitational field where space-time is flat (since the Riemann curvature tensor vanishes).

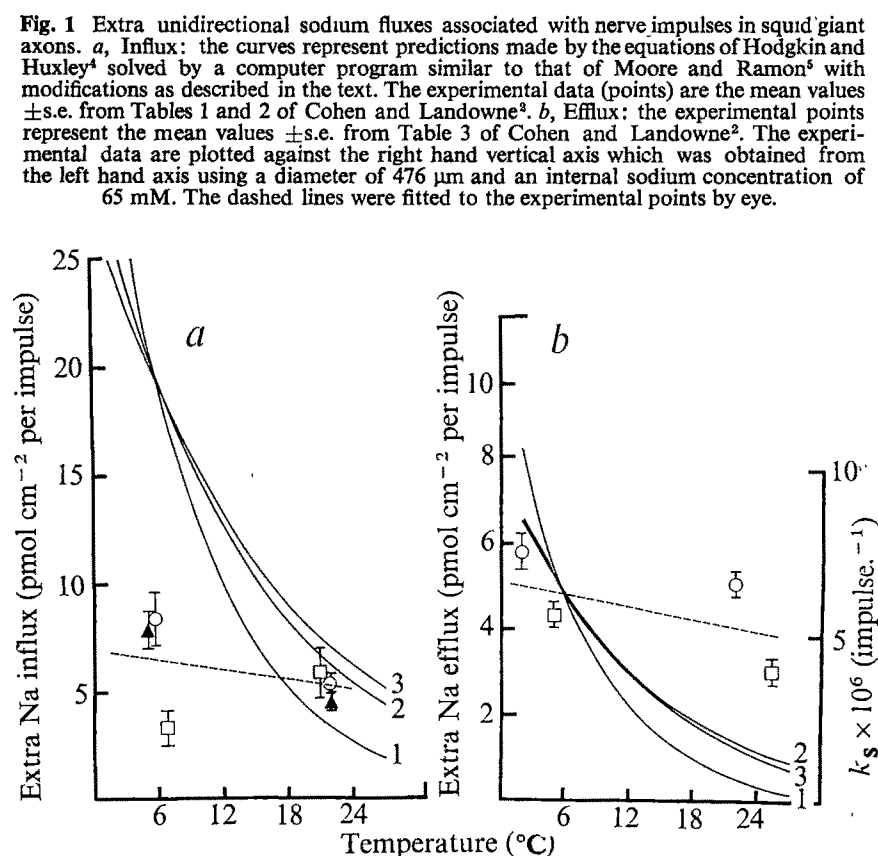
Einstein's 1911 paper is not, of course, in the framework of Newtonian physics. The argument

Equivalence principle

→ Retardation of clocks

cannot be applied in Newtonian physics because of the existence of an absolute time. The principle of equivalence itself is only partly contained in Newtonian physics in that "fictitious forces" can be equated with gravitational forces. Since the equivalence principle requires, in addition, that the behaviour of clocks under the influence of a gravitational field and an (locally equivalent) acceleration field be indistinguishable at a point, it may not be fully incorporated into Newtonian physics.

The redshift which arises in the treat-



ment of accelerated motion requires only the use of the Doppler effect and is merely equivalent to the statement of conservation of energy⁵. Only in special relativity can one equate a Doppler shift with clock retardation which is necessary so that all inertial observers measure the same value for the velocity of light. The principle of equivalence extends this to all freely falling observers.

Furthermore, it can be argued that the full principle of equivalence would be inconceivable before the advent of special relativity: as long as one believed that the velocity of light depends on the motion of the source and/or of the observer, how could one equate an accelerated system with one at rest but in a gravitational field?

We agree with Bishop and Landsberg that relationships between inertial and non-inertial frames are not suitable for discussing the gravitational redshift, and, in fact, this lies at the basis of our treatment in ref. 3.

G. E. MARSH

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C. NISSIM-SABAT

Northeastern Illinois University,
Chicago, Illinois 60625

¹ Bishop, N. T., and Landsberg, P. T., *Nature*, **252**, 459 (1974).

² Kilmister, C. W., *Nature*, **252**, 439 (1974).

³ Marsh, G. E., and Nissim-Sabat, C., *Am. J. Phys.*, **43**, 266-267 (1975).

⁴ Schild, A., *The Monist*, **47**, 20 (1962).

⁵ Misner, C. W., Thorne, K. S., and Wheeler, J. A., *Gravitation*, 187 (Freeman, San Francisco, 1973).

As one of the authors referred to by Bishop and Landsberg¹, I would like to comment before students abandon their text books. Bishop and Landsberg¹ recommend that one should not use Newtonian mechanics with the principle of equivalence, to develop the gravitational redshift. They say that the result "is an absurd conclusion, since Newtonian physics operates with an absolute time". The principle of equivalence also leads to the result that the path of light is curved in a gravitational field. Thus, in another context, it is equally absurd to use the principle of equivalence with special relativity, since light signals are used to synchronise clocks and mark out straight lines in special relativity. Thus, in the presence of gravitational fields, strictly one should only use the general theory. What is done in elementary text books is to add the principle of equivalence on to either Newtonian mechanics or special relativity, whichever is the more convenient. It is an extension in both cases. It serves only as an introduction to the general theory.

A typical approach², considers a light source and detector at the rear and front ends respectively of an accelerating spaceship. The spaceship is considered from

the inertial reference frame in which it is instantaneously at rest, when the light is emitted. Both source and detector have an acceleration a in this frame. The proper length l of the spaceship can be measured using a ruler at this instant. The signal takes a time $t \approx l/c$ to reach the detector, by which time the detector has a speed $v \approx at \approx al/c$ relative to the inertial frame. Provided $v \approx al/c \ll c$, the first order Doppler effect, given by the wave theory of light, is adequate, so that the fractional change in frequency $\Delta v/v \approx v/c \approx al/c^2$.

According to the principle of equivalence, the difference in frequency should be the same, if the spaceship were at rest in a gravitational field of intensity $-a$. Provided $al/c^2 \ll 1$, it is an unnecessary over-elaboration to use the equations of hyperbolic motion, and in a first introductory course the use of Newtonian mechanics and the first order Doppler effect, based on the wave theory of light, is far more convenient. In practical cases $\Delta v/v \sim 2 \times 10^{-15}$ in the experiments of Pound and Rebka and $\sim 2 \times 10^{-6}$ for light from the Sun, so that $al/c^2 \ll 1$, and the simple theory is accurate enough. Near black holes one would have to use the general theory of relativity. This approach, based on the principle of equivalence, should only be treated as a teaching aid, to give a first insight into some of the modifications of both Newtonian mechanics and special relativity required by the general theory of relativity.

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¹ Bishop, N. T., and Landsberg, P. T., *Nature*, **252**, 459 (1974).

² Rosser, W. G. V., *Introductory Relativity*, 264 (Butterworth, London, 1967).

³ Pound, R. V., and Rebka, G. A., *Phys. Rev. Lett.*, **4**, 337 (1960).

BISHOP AND LANDSBERG REPLY—We shall explain the points raised by referring to the paragraphs in ref. 1 as $a-e$, those in ref. 2 as $a-c$ and those in ref. 3 as $a-g$. We referred^{3b} to a non-Minkowskian metric. This does not imply that we considered the uniform acceleration to produce space-time curvature^{1a}. We had in mind flat (that is, $R_{\rho\gamma\delta} \equiv 0$) non-Minkowskian metrics of the type⁴

$$ds^2 = g_{44}(x)c^2 dt^2 - \frac{c^4}{4\kappa^2 g_{44}} \left(\frac{dg_{44}}{dx} \right)^2 dx^2 - dy^2 - dz^2$$

We thus agree with Marsh and Nissim-Sabat^{1a} that the existence of a gravitational redshift does not imply space-time curvature, but we do not accept that any

such curvature is suggested by us^{3b}. Rosser^{2a} regards curved light ray paths, and therefore accelerated frames, as unacceptable in special relativity. We^{3d} take the opposite view, which is implicitly supported by Marsh and Nissim-Sabat^{1a}.

For the Newtonian case we used a particle picture of light^{3d} in which light particles are emitted with a constant speed relative to the source, and move according to Newton's laws of particle mechanics. The equality of inertial and gravitational mass leads to the equivalence principle for particles, and thus also for Newtonian optics. From our point of view, therefore, the equivalence principle is completely contained in Newtonian physics, as opposed to special relativity where the principle has to be grafted on, since this theory is not about gravity. Our correspondents disagree with this view (and with each other), the view of Marsh and Nissim-Sabat^{1b} being closer to us than that of Rosser^{2a}. For a wave theory of light (advocated in ref. 2c) the velocity of light is constant with respect to an ether and the equivalence principle does not then apply to Newtonian optics, as also remarked by Marsh and Nissim-Sabat^{1a}. The answer to the question raised there is: "By using a particle picture of light".

An important point of our letter was the view reiterated by Marsh and Nissim-Sabat^{1e} but not taken account of by Rosser^{2b}: the replacement of a uniform gravitational field by a uniform acceleration for source and detector is always magically transformed to a Doppler effect argument for an accelerated detector, thus neglecting the motion of the source. This is a non-trivial step since two times are needed at the source if a frequency is to be defined there. Thus the switch from one model to the other has to be justified, and if presented clearly to beginners^{2c} ought to elicit from them a request for a proof. Our work⁴ may be regarded as providing such a proof for special relativity and, in so far as this had never been done in the past, one was faced by a misuse of the equivalence principle. For Newtonian physics we show⁴ that it is this magical switch in the usual argument that leads apparently to the absurdity of clock retardation by way of the Newtonian Doppler effect. As developed by us⁴, one finds a zero Newtonian gravitational redshift but a non-zero Newtonian Doppler effect for an accelerated detector, and the usual equations for special relativity. These results seem to us clear and consistent.

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¹ Marsh, G. E., and Nissim-Sabat, C., *Nature*, **257**, 517-518 (1975).

² Rosser, W. G. V., *Nature*, **257**, 518 (1975).

³ Bishop, N. T., and Landsberg, P. T., *Nature*, **252**, 459 (1974).

⁴ Landsberg, P. T., and Bishop, N. T., *Foundations of Physics* (in the press).

reviews

IN the study of many subjects, including geography, psychology, statistics, eugenics and meteorology, the name of Francis Galton receives mention from time to time. But few, in their training, receive more than a glimpse of the life and work of this somewhat elusive man. Who was he? Where can one gain a comprehensive picture of him? A visit to the library soon leads to the biography by Karl Pearson, completed in 1930, but this work (2,000 pages) is too much for many readers to face. Thus, for many he is still not within reach. But now the position changes, with the timely publication of this book, and at last we have a manageable and readable account; and one which, although only one-sixth of the length of Pearson's work, is nevertheless much more objective.

The first chapter is concerned with the family background and the life of Galton up to the age of 22 (when his father died). Succeeding chapters describe Galton's years as an explorer, in Egypt, Syria and South West Africa, and contain some fascinating accounts of his experiences. One result of these explorations was the production of the famous book entitled *The Art of Travel*, a book which was of great practical value to those contemplating travel in unexplored territory.

After the years of exploration Galton had much to do with the Royal Geographical Society, and with the development of geography as an academic subject; and he played a large part in having the subject introduced at

Francis Galton: psychology of a polymath

Francis Galton: The Life and Work of a Victorian Genius By D. W. Forrest. Pp. x+340+16 plates. (Elek: London, November 1974.) £5.50.

Oxford University. He also became interested in meteorology, and here he demonstrated his capacity for original scientific research. He studied weather data brought back from Africa by explorers and this led to the discovery of the anticyclone. In 1863 he published a book, *Meteorographica*, containing hundreds of diagrams of climatic conditions in Northern Europe during December, 1861.

By the mid 1860s Galton began to develop an interest in heredity (there is some evidence well documented in this book that the development coincided with a growing realisation that his marriage would probably be infertile). Two chapters carefully trace this interest.

Galton was to a large extent the father of present-day psychology, and several chapters cover in detail his many ideas and experiments in this

field. He was also a pioneer in the development of anthropometry and, at his own expense, set up an anthropometric laboratory as part of the International Health Exhibition held in 1884. His work in this field proceeded with much vigour during the following years and an outstanding development was the technique of personal identification using fingerprints. This work is treated in a chapter devoted to the subject. In 1883, Galton published his last important technical book, *Fingerprints*.

In May 1904 Galton delivered a lecture (at the London School of Economics) to the newly formed Sociological Society, entitled "Eugenics, its Definition, Scope and Aims". This was the birth of the subject of eugenics, and Galton contributed much to the development of this subject. He was by this time, however, into his eighties and his health was on the decline. He died in 1911 at the age of 89.

Professor Forrest's book will be of interest to students drawn from various disciplines. It includes a detailed account of Galton's inventions of various pieces of apparatus, and a bibliography of his published works. It reveals a great deal about the psychology of one who pioneered a good deal of modern psychology (especially with emphasis on the experimental approach), and also a lot about Victorian society in general. The book will be of interest to historians as well as psychologists, biologists and statisticians.

L. S. Goddard

NUCLEAR form-factors measured in elastic electron-scattering experiments decrease with increasing momentum transfer at a rate determined by the nuclear size. If one looks instead at the inelastic region, where the electron loses large amounts of momentum, q , and energy, ν , the cross-section is dominated by quasi-elastic scattering from the individual nucleons in the nucleus, and the q -dependence is a measure of the proton size. In analogous experiments with a hydrogen target, Stanford physicists claimed that in this deep inelastic region they could 'see' the constituents of the proton itself; and the q -dependence indicated that these partons were point particles. Bjorken scaling was observed; apart from kinematic factors the cross-section in this region depended only upon ν/q^2 rather than the independent variables.

Kindergarten parton model

Theory of Lepton-Hadron Processes at High Energies: Partons, Scale Invariance, and Light-Cone Physics. (Oxford Studies in Physics.) By Roy Probir. Pp. x+172. (Clarendon: Oxford; Oxford University Press: London; February 1975.) £6.

Some five years later this "kindergarten parton model" is still very much with us, and Dr Roy's book is a description of these intuitive ideas with their experimental consequences, and the more formal concepts, of scale invariance and

light-cone algebra which have developed from them. The book is likely to be useful to second-year graduate students and 'above'; in the early part it requires knowledge of simple quantum electrodynamic calculations but towards the end the renormalisation group in field theory is called for.

Any introduction to a rapidly changing field can expect to be overtaken by events. Thus, if the author had written the book after the discovery of the ψ particles last November, he would doubtless have stressed the charm quantum number much more. Scaling has influenced the interpretation of deep inelastic proton-proton scattering, but the author has wisely confined himself to lepton scattering where a basic formalism exists and is likely to be of some lasting value.

Colin Wilkin

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Heavy nuclei

Heavy Nuclei, Superheavy Nuclei, and Neutron Stars. (Oxford Studies in Nuclear Physics.) By J. M. Irvine. Pp. 158. (Clarendon: Oxford; Oxford University: London, July 1975.) £8.

THE prospective reader who is attracted to this book by the words "superheavy nuclei" or "neutron stars" is likely to be disappointed. Of its 158 pages of text only one chapter of 8 pages is concerned directly with superheavy nuclei, and a further 9-page chapter is devoted to neutron stars. What it does contain is an excellent review of the current theories of the properties and structure of heavy nuclei.

The book concentrates on the region of heavy nuclei beyond ^{208}Pb and as is pointed out in the introductory chapter this contains only ten naturally occurring elements, although a further 14 have been artificially produced in the laboratory. It is perhaps disappointing that the book contains no hint of the excitement and controversy which have surrounded the discovery of these heaviest elements.

The second chapter contains a brief but thorough review of the observed systematics of heavy nuclei such as binding energies, half lives,

decay modes and fission properties. The third chapter then goes on to give a very detailed discussion of the nuclear models, such as the liquid-drop and individual particle models, which have been used to explain these systematic features. Nuclear correlations are introduced here and used to bridge the gap between these two very simple models. The following chapter deals with the particular topic of collective rotations and the specific modifications which need to be considered to bring theoretical predictions into quantitative agreement with experiment.

Nuclear stability and shell effects are next considered and this leads very naturally, through a discussion of fission and alpha decay, to the prediction of the possible existence of superheavy nuclei and the final chapter on neutron stars. A short list of references to relevant review papers and books is included.

The book is written at an introductory level suitable for research students in nuclear structure physics. It is also likely to be of interest to more experienced workers in this field as providing an extensive and thorough review of current theories of the properties of heavy nuclei with a hint and hope of discoveries yet to come.

C. J. Batty

Come back Aristotle

Confrontation of Cosmological Theories with Observational Data. Edited by M. S. Longair. (International Astronomical Union Symposium No. 63, Cracow, Poland, 1973.) Pp. xi+382. (Reidel: Dordrecht and Boston, Massachusetts, 1974.) Dfl.115; \$38.50.

THIS symposium, held in 1973 to commemorate the 500th anniversary of the birth of Copernicus, provides an excellent picture of cosmology today—of a science caught, as Zeldovich says in his introduction, at a turning point. The programme of Sandage and Tammann to extend the redshift-distance relation for galaxies, the close agreement between the expansion time of the Universe and the age of our Galaxy and other galaxies, and the 2.7 K blackbody spectrum and high degree of isotropy of the microwave background radiation (reviewed here by Blair, Partridge and Boynton) have together convinced all but the most sceptical cosmologists that we live in a hot, big bang Universe.

In paper after paper the most simple, conservative view seems to be supported. Petrosian argues that the cosmological term, introduced and later repudiated by Einstein, is zero. Tammann demonstrates that the velocity flow field of nearby

galaxies is isotropic and linear. Wagoner shows how the cosmic abundances of those elements and isotopes formed primarily in the fireball (deuterium, helium, lithium) favour the simplest type of model, deuterium, in particular, pointing to the existence of the low-density Universe also indicated by the density of matter in galaxies. Steigmann sets severe limits on the amount of antimatter in the Universe.

In his amusing introduction Zeldovich sets out what seems to be a very open minded philosophy of cosmology. That we cannot simply solve backwards in time the equations describing the evolution of the Universe, but have to take arbitrary chosen variants of the initial state and follow the theory of its evolution to the present and thus to a confrontation with observations. The snag of this procedure being that it is dependent on the prejudices, likes and dislikes of authors and "perhaps even their subconscious Freudian attitude to such things as order, chaos, antimatter". Hence the importance of the confrontation with observation, in which "false theory fades".

But is it really cosmological theories that are being confronted with observations? Apart from a few dissenting, sceptical and dissatisfied voices (for example, those of Arp, Kellermann,

Omnes and Elvius), only one theory is discussed in this book. Kuhn's gestalt switch has occurred and the isotropic big bang has become the paradigm. Within that framework the theoreticians set to work on the details: the problem of galaxy formation (Silk, Doroshkevich, Sunyaev, Zeldovich and Ozernoy), the structure of the initial singularity (Penrose, Novikov, Lifschitz and Khalatnikov), and the exciting work on pair creation by anisotropic expansion (Zeldovich and Misner). This is a situation in which the mathematically brilliant but philosophically conservative Russian school of cosmology flourishes. But is it likely that we shall again have to wait the 2,000 years between Aristotle and Copernicus to see this picture overthrown?

Yet even in the ranks of the believers there are some novel perspectives. Hawking analyses homogeneous, anisotropic perturbations of the standard models and concludes that we must be in a unique model (with zero curvature), arguing that the reason for this must be, in a sense, because we are here. If the Universe was not as it is, galaxies and stars could not have formed and we would not be here to observe it—the 'anthropic principle' developed elegantly in a paper by Brandon Carter.

Come back Aristotle, all is forgiven.

M. Rowan-Robinson

Complex uses of an advanced tool

Quantitative Scanning Electron Microscopy. Edited by D. B. Holt, M. D. Muir, P. R. Grant and I. M. Boswarva. Pp. x+570. (Academic: London and New York, January 1975.) £15.50.

SINCE the early 1960s, the accessibility of the scanning electron microscope (SEM) as a working tool in the hands of scientists has grown tremendously. For example, just one of the several SEM manufacturing companies lists over 500 owners throughout the world, which may imply 10 times as many users. Furthermore, a lot of ingenuity has gone into finding new uses for a tool which yields dramatic topographical pictures suitable for hanging on the wall but can also probe the basic electrical and crystallographic properties of materials in many more subtle ways. This book attempts to review the unique methods by which the properties of inorganic materials may be measured quantitatively using the interaction of a rastered kilovolt electron beam with a solid. It explicitly avoids an introductory approach, but, as parts of it evolved from a lecture course, it is well adapted for an advanced teaching course.

Fifteen authors, mainly from British universities, gave authoritative reviews of each use of the SEM and a strong quadripartite editorial team has successfully imposed unity on the contributions, keeping a sharp eye on the possible future development of the instrument. Several aspects of the production of finer and better controlled electron beams occupy the first three chapters, including an unusual and useful review of the very complicated interaction of electrons with solids and a rather casual run through "the approach to 1 Å" by the renowned Albert Crewe. Six chapters on the quantitative interpretation of contrast borrow from and build upon the art developed for the transmission electron microscope (TEM). Howie's chapter does this by means of a unified survey of diffraction contrast and scattering in the TEM mode (including the scanning and glancing-angle modes) and the backscattering mode.

The emissive mode, for all its importance, is compressed efficiently by Gopinath into only 30 pages, but it would not have been too much of a diversion for the editors to have inserted a chapter on the morphology and physics of crystalline organic materials, thereby capturing a large additional readership.

Finally, the versatility of the SEM is suitably revealed in the section on the use of the X-ray yield of the SEM

sample. This section again gives prominence to creative, forward-looking aspects and, indeed, the final chapter on automatic stereological analysis by Jones indicates that a strong use of the imagination and the help of a computer can tell one what is in the bulk of a mineral conglomerate, working only from the surface X-ray energy analysis. The greatest lack I noticed was of any discussion of the positive side of electron beam damage such as is observed with kilovolt electrons on films of alkali halides. In fact, the editors have stuck rather narrowly to the technologically useful metals, semiconductors and phosphors and the emission therefrom.

This book is not a handbook; I regret somewhat the lack of survey tables and appendices. In general, the editors have not pressed their authors to prepare the information better for their readers. Diagrams are redrawn from the research literature rather than pulled apart, condensed and recombined for clarity. The subediting on diagrams is sometimes loose as to symbols and captions, which may cause the neophyte some crises of confidence. These minor points aside, this is a book which the novice microscopist having grasped the basic principles elsewhere, can use with confidence as a guide to the complex uses of the SEM.

Andrew Holmes-Siedle

Maternal lifeline to foetus

The Placenta and its Maternal Supply Line: Effects of Insufficiency on the Fetus. Edited by P. Gruenwald. Pp. x+366. (MTP: Lancaster, April 1975.) £11.50.

THE declining perinatal mortality in developed countries has highlighted the importance of foetal subnutrition *in utero* as an increasingly common cause of perinatal morbidity and mortality. The present volume is therefore well timed and provides a useful summary of current theories concerning the physiology of foetal growth and possible therapeutic approaches to suboptimal growth patterns *in utero*. Professor Gruenwald has a distinguished record in this area, and was one of the first workers to stress the difference between infants of low birth weight due to premature delivery and those due to intra-uterine growth retardation. The volume consists of chapters written by a variety of contributors—usually

research workers with a particular interest in the subject—and there is therefore no uniform style. Many of the chapters are well illustrated, whereas some have no illustrations at all. The illustrations used by the editor tend to be complicated and difficult to interpret at a glance. The majority of authors present an objective view of their subject but in some cases the author's own viewpoint is emphasised with only cursory reference to opposing schools of thought. The book is well referenced and the bibliography is extensive.

On balance the volume presents a comprehensive review of the current literature on placental development and pathophysiology, as well as a shorter section on foetal growth and pathophysiology, not only in human but in other species. The authors emphasise certain areas in which further research is needed, and particularly the need for new diagnostic and therapeutic possibilities in pregnancy. At £11.50 the volume is recommended as a valuable addition to every medical and biological sciences library as well as to interested investigators.

Y. B. Gordon

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obituary

John Ray Dunning, a pioneer in the development during World War II of the method of isolating uranium 235 used in nuclear weapons and power plants, has died at his home in Florida. He was 73.

Dr Dunning started his graduate career at Columbia in 1929 and was appointed assistant professor in physics in 1935. He toured many European nuclear physics research establishments during the following year and was to become associated with Bohr and Fermi on his return to America. He directed the development of Columbia's first cyclotron, built in 1936, which was to be used in the university's history-making experiments. In determining that uranium 235 is the isotope which readily undergoes fission into

two atoms of nearly equal size and that the reaction releases prodigious amounts of energy, Dr Dunning helped to lay the foundations of the atomic age. He was essentially confirming the results of the enemy in Germany, but his experiments were nevertheless of paramount importance in winning the race to production. He was promoted to full professor in 1946 and in that year served as special representative for the Manhattan District at the first postwar detonation of an atomic bomb on Bikini Atoll. During 1941-46, he was an official investigator of the Office of Scientific Research and Development. From 1942-45 he was director of research in the substitute alloy materials laboratories, which was the official name for the secret gaseous

diffusion project to isolate uranium 235 from 238 for the production of atomic bombs (this process is still the major source of such "fuel"). In 1945 he was appointed director of Columbia's division of war research, and the following year was awarded the Medal of Merit, the highest award made by the US President to civilians. In 1950 he saw the completion of the university's 385 million electron volt synchrocyclotron at Nervis, New York after serving as scientific director of the project.

After 19 years as dean of the faculty of engineering, during which time he had raised over \$50 million for the research effort, he resigned to become Thayer Lindsley Professor in applied science in 1969.

announcements

International meetings

December 1-2, **DNA-repair and late effects**, Vienna (International Atomic Energy Agency, 1010 Vienna, Kartner Ring 9, Austria).

December 1-5, **Nuclear techniques in exploration extraction and processing**, Rio de Janeiro, Brazil (International Atomic Energy Agency, 1010 Vienna, Kartner Ring 9, Austria).

December 2-3, **Grass weeds**, Paris (M. R. Faivre-Dupaigre, 8 Avenue du President Wilson, 75116 Paris, France).

December 3-5, **Cardiology**, Houston (The Office of the Director, The University of Texas Health Science Center at Houston, Division of Continuing Education, PO Box 20367, Houston, Texas 77025).

December 11-12, **Recent advances in chemistry and technology of fats and oils**, Liverpool (Head of Chemistry Department, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, UK).

December 16-18, **Structure and function of membranes**, London (Dr J. C. Metcalfe, Department of Pharmacology, University of Cambridge, Medical School, Hills Road, Cambridge CB2 2QD).

Reports and publications

Other Countries

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The Winged Bean: a High-Protein Crop for the Tropics. Pp. vii + 41 (Washington, DC: National Academy of Sciences, 1975.) [158]

Records of the Australian Museum. Vol. 29, No. 12. The Alpheid Shrimp of Australia. Part 2: The Genus *Synalpheus*. By Dora M. and Albert H. Banner. Pp. 267-389. 50c. Vol. 29, No. 18. *Periclimenella colemani* SP. NOV., a New Shrimp Associate of a Rare Sea Urchin from Heron Island, Queensland (Decapoda Natanitina, Pontoninae). By A. J. Bruce. Pp. 485-502. 50c. Vol. 29, No. 19: Australian Species of *Haliophasma* (Crustacea: Isopoda: Anthuridae). By Garry C. B. Poore. Pp. 503-533. 50c. (Sydney: The Australian Museum, 1975.) [158]

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nature*October 16, 1975*

Peace is too politicised for prizes

THE award of the Nobel Peace Prize to Academician Andrei Dimitrievich Sakharov took nearly everyone by surprise. No doubt he could at one time have been considered a candidate for a Physics Prize, but few had thought of him in the context of a Peace Prize. So was the action of the Oslo committee an inspired gesture aimed at expanding the horizons of 'peace' to include human rights as the "only sure foundation for a genuine and long-lasting system of international cooperation"—as the citation read; or was it simply a political act, such as can be carried out from the relative safety of Scandinavia, designed to cause a certain amount of embarrassment to the Soviet Union?

First, let it be said that Sakharov's record, initially as a physicist, then as a proponent of restraint in weaponry and finally as a social democrat fighting for civil rights has been admirable (see page 528). If the year's award is controversial, the controversy is not centred around the personal qualities of Academician Sakharov in any way. Furthermore, it is undeniable that the award of this prize will have done much to encourage Sakharov, if encouragement were needed, to continue his work.

So far so good. But even if the West can, on the whole,

view the award as given for human rights work, the Soviet Union undoubtedly views the award as a piece of political cynicism supportive of troublemakers. If the Nobel Foundation were a political outfit devoted to the supporting of Western ideals that would matter little—but Nobel Prizes would then have the significance only of Stalin Prizes. By aspiring to have a global significance Nobel Prizes must be free of the sort of ambiguity and polarisation that this year's prize generates; and not only this year's—recent awards to Willy Brandt, Le Duc Tho and Henry Kissinger have all in their different ways been political and arguable.

If this means that it is impossible to find globally acceptable recipients of Peace Prizes, then perhaps this is simply a recognition of the fact that the concept of peace is highly politicised and ambiguous, and it is poorly served by the handing out of large sums of money to individuals.

We have noted before that Nobel Prizes often leave a trail of devastation behind them. This is particularly so of the Peace Prize; it is time that the Nobel Foundation seriously looked at other ways of supporting its very worthy ideals.

Better news from the universities

EVEN the most bashful of Britain's universities are now back in business for a new academic year and, according to figures released last week, the population of full-time students (including graduates) is up 10,000 to 261,000. First-year students are up in number by 7%. The reason for the continued growth (albeit at a slower pace than was once predicted) is not entirely that there is a still-growing demand for more higher education among the nation's young. Many of this year's intake will be somewhat reluctant students; the economic situation is persuading many school-leavers that three years at university would be safer than unemployment at 18.

In the past two years universities have been in the forefront of the government's cost-cutting campaign and as a result are now 5% worse off in real terms than they might have expected to be. Sir Arthur Armitage, Chairman of the Committee of Vice-Chancellors and Principals, in announcing these figures, called the meeting of increased demand and the maintenance of university education under these circumstances "no mean contribution to public policy". Few will disagree with him, and

many will hope that the so-called fat-trimming exercise is now at an end.

Behind the overall figures are two statistics; one encouraging, one less so. Even though there are still 10,000 empty science places, there is good evidence that the swing away from the natural sciences has been halted. On the other hand the postgraduate scene is still very static and there might even in the near future be such a thing as a shortage of people with postgraduate degrees. The proportion of overseas students in the postgraduate population remains high.

With 300 university teaching posts now frozen for lack of funds, it is incumbent on universities to enunciate a policy for when (if ever) there is a thaw. There has been a strong element of chance in the freeze—departments with staff at retirement age have been particularly vulnerable. But it is not necessarily logical simply to put the same posts back on the market. A crisis of this dimension offers an excellent opportunity for change in a system normally gripped by tenure. It would be foolish to throw up such an opportunity.

THE 30th Report from the Select Committee of the House of Lords on the European Communities is a slender pamphlet which at first glance seems too trivial to merit a column in *Nature*. It comments on two draft directives from the EEC: one about pollution on bathing beaches (an unseasonable topic anyway, at this time of year), and the other about pollution from wood pulp mills, of which there are only seven in Britain.

In fact the report is anything but trivial. It raises a matter of high principle in Britain's relations with the EEC, affecting our whole policy for the control of pollution. On October 13, the House of Lords "took note" of the report and there was a short debate on it. The debate ought to go on outside Parliament as well.

The matter of principle is this. The Council of the European Communities agreed, in a declaration on November 22, 1973, that "improvement in the quality of life and the protection of the natural environment are among the fundamental tasks of the Community". Admirable. No-one would dissent from this. It follows that there must be a policy among the member states for the abatement of pollution. This policy is now being worked out through numerous draft directives from Brussels prescribing standards for car emissions, lead in petrol and crockery, sulphur in oils and, most recently, the pollution per tonne of product from pulp mills and the quality of water in which bathing is "authorised or tolerated".

The philosophy underlying these directives is to fulfil the 1973 Declaration by imposing identical and uniform standards upon all member states, from Italy jutting into the Mediterranean to Scotland jutting into the Atlantic, irrespective of the diversities in the environments of these states. There is a Gallic elegance and simplicity about this, but it is quite inconsistent with the pragmatic policies which determine the procedures for pollution control in Britain. Do we stick to our present practice and persuade the EEC to accept it? Or do we submit to these inflexible directives from the Community?

Before arguing this point we have to distinguish three kinds of standard, for this controversy with the EEC is concerned with only one of these three kinds. There is a good case for uniformity over product and design standards (for example, the level of pesticide residues in foodstuffs and the emission standards from car exhausts) because food and cars move from one part of the Community to another. There is also a case, provided it is not made too rigid, for long term objectives for the purity of the air and of

the sea. The controversial question is whether there should be Europe-wide identical standards for the quality of emissions into the air from smoke stacks and of effluents into rivers and the sea from outfalls. In other words, must specifications for the discharge of wastes into the environment be identical irrespective of the capacity of the environment to disperse or dilute the wastes?

The practice in Britain is empirical. The consents to discharge effluents, issued by a Regional Water Authority, depend on the capacity of the river or estuary, the uses to which it is being put, the pollution load it is already carrying and so on. The licence to discharge "noxious vapours" into the

A community of interests?



ERIC ASHBY

air, issued by the Alkali Inspectorate, depends on the best practicable means for controlling the vapours at the time the works was erected, the cost of installing new equipment, the economic state of the industry and so on. In brief, the British "philosophy" (if one can call it that) is not to minimise pollution, nor to bring all discharges of any pollutant down to some uniform level (though there are guidelines for quality standards for emissions): it is to optimise pollution levels so that the capacity of the environment to accept (and in many cases to recycle) pollutants is fully used, but not abused, and industry (and thus employment) are not unduly put at risk. (Rabid conservationists need to be reminded, sometimes, that all pollution except that from atomic weapons is a by-product of processes which benefit society.) This "philosophy" can be defended on two counts: it makes the best use of the limited resources available for the control of pollution; and it acts (as the Alkali Inspector put it in a report published in 1888) as "an

elastic band ever tightening as chemical science advanced". Thus in the sulphuric acid industry in Britain there are plants emitting 5%, 4%, 2% and now 0.5% of the sulphur burnt. These different levels are related to the best practicable means for control at the time the plants were built. Had we been obliged to adopt a uniform emission standard it would either have been too lax for new works or it would have added unreasonably to the costs of running older works.

Consider now the EEC's draft directive for effluents from wood pulp mills. It lays down that within 10 years pollution shall be reduced to levels set out in the directive. These prescribe the biological oxygen demand (BOD) and suspended solids (SS) per tonne of product, above which discharges from mills will not be permitted. There is an escape clause which allows derogations from these standards if the discharge is into tidal waters, provided the discharges "do not cause an appreciable deterioration in the quality of the receiving water" ("appreciable" is not defined!). But the derogations expire after a maximum of 10 years. So this is what the directive means: that ultimately the permitted effluent standard from a wood pulp mill shall be identical, whether the mill discharges into the Thames or the Rhine or into the Atlantic Ocean off the Scottish or the French coast.

It requires no subtlety to see that this sort of rigidity would be a wasteful way to deploy the limited resources we have for the abatement of pollution. But the EEC has another reason for pressing uniformity on member states. It asserts that if the Scottish mill discharging into the Atlantic can get away with less stringent effluent standards, this will constitute unfair competition against the mill on the Rhine, which has to comply with very stringent standards. This is a phoney argument, for you might as well say that the wage rates and the transport costs for the Scottish mill will constitute unfair competition unless they, too, are identical with the rates and costs for the mill on the Rhine. "Harmonisation" of practices (as the EEC calls it) among member states is obviously a sensible objective, but it becomes irrational if it disregards the great diversity of environments in the Community.

The draft directive for the quality of bathing water is unacceptable for a different reason. It is not that we are content to bathe in dirtier water than our common market cousins bathe in; it is that a code of control which might be appropriate on a Mediterranean beach at a temperature of 27 °C would be inappropriate on an Aberdeenshire beach at a temperature

(under favourable conditions) of 10 °C. The EEC directive goes into great detail about the monitoring of beaches where bathing is to be authorised or tolerated. (How does one deal with a bather who persists in bathing in a place which is not tolerated? The directive is silent on this point.) Monitoring must be carried out for each beach once a week, or once every two weeks, or once a month, depending on "the mean density of bathers per kilometre of beach" and bathing is defined as "prolonged immersion of the whole body". Bathing water is "deemed to conform to the mandatory values of the relevant parameters if 95% of the samples . . . comply with the limits specified in column I of annexes 1 and 2". Now turn to the list of parameters in the annex. They include: total coliform, faecal coliform, faecal streptococci, *Salmonella*, viruses, pH, mineral oils, surface active substances reacting with methylene blue, phenol indices, pesticides, and the following: As, Cd, Cr, Pb, Hg, CN, NO₃, phosphates and dissolved oxygen. Consider the task of carrying out these acts of monitoring, even for the major watering places round the coasts of Britain. The Department of the Environment hazarded a guess that the cost of complying with the directive might be of the order of £100 million a year. If there were a demonstrable health hazard this enterprise might be justified, but such data as we have (from a Medical Research Council report published in 1959) indicate that although many beaches in Britain are offensive to the eye and the nose, they are not a hazard to health; and the WHO still takes the view that "there are no internationally accepted criteria for the quality of coastal water for bathing with respect either to microbial contamination or chemical pollution".

There are no grounds for complacency about the state of the environment in Britain. We are certainly not in disagreement with the EEC's Declaration to protect the environment and we have, in the Control of Pollution Act, the best legislative arrangements in Europe for doing the job. The matter of principle, on which I hope Britain will stand firmly, is that each member state should be free to fulfil the objectives of the Community's environment policy in the light (as the Declaration states) "of health and ecological requirements"—that is to say, taking into account the uses to which the environment is being put, and its capacity to dilute or disperse wastes. This is the basis of our pragmatic style of pollution control. This is the style we shall have to abandon if we cannot persuade the EEC to make its directives more flexible. □

Candle power at Browns Ferry

In his third article on the state of the US nuclear industry, Colin Norman recalls the bizarre and dangerous accident at a nuclear power station earlier this year.

A FIRE, started by an electrician with a candle, disabled the Browns Ferry nuclear power station in Alabama on March 22, 1975, and sent shock waves through the nuclear industry. Officially described as the most serious accident to occur so far at an operating nuclear power plant in the United States, the fire knocked out several key safety systems, caused problems in shutting down one of the plant's two reactors, and rekindled the long-smouldering debate about nuclear safety.

A bizarre and dangerous accident which destroyed some 2,000 electric cables and caused millions of dollars worth of damage, the fire has put the Browns Ferry power station out of action for an indefinite period. It has also raised a number of serious questions about the vulnerability of nuclear power plants to such unforeseen incidents, and it may result in some costly modifications to other nuclear power stations in the United States.

The nuclear industry has, however, managed to salvage some good news from the incident. Although several key safety systems were disabled by the fire, enough reserve systems were left intact to shut the plant down safely and to avoid a major accident. That, industry spokesmen suggest, supports their contention that there are so many safety features built into a nuclear power station that if one device fails, others will be left to take over if necessary.

The Nuclear Regulatory Commission (NRC), the agency responsible for regulating the nuclear industry and ensuring that nuclear power plants are safe, shares that interpretation. In testimony before the Joint Committee on Atomic Energy last month, for example, the Chairman of the NRC, William A. Anders, said "we believe that this unfortunate and serious occurrence has shown that the reliance on the defence-in-depth concept is sound for the protection of public health and safety".

The NRC is now in the midst of analysing the Browns Ferry fire and its implications. It has already published a factual report on the accident, and in the next few weeks, the NRC will

recommend changes in design and operating procedures at other nuclear power stations to prevent similar incidents from occurring elsewhere. Its report on the fire itself is a disquieting document which reveals an astonishing lack of fire precautions, considerable confusion during the fire fighting operations, and some quick thinking in the control room to avert a major nuclear disaster.

The fire began at about 1215 on March 22, when two reactors at Browns Ferry were each generating about 1,100 MW of electricity. A third reactor, of similar size, was under construction. The fire was touched off by two electricians who were testing for air leaks around cables passing through the wall of a room enclosing the Unit 1 reactor.

The air in the reactor room is maintained at a lower pressure than the air outside, so that if radioactivity were released from the reactor, it would not immediately be dispersed into the atmosphere—fresh air would tend to leak into the reactor room rather than contaminated air out of it. The electricians were checking for leaks in a part of the wall separating the reactor room from a cable spreading room, immediately beneath the reactor's control panels. They were using the highly unofficial but time-honoured method of holding a lighted candle to the outside of the wall at the point where cables pass through it—if the candle flame is sucked horizontally, air is leaking from the cable spreading room into the reactor room.

An electrician's mate, who was doing the candle work, told NRC investigators that a fire started when the candle flame was sucked into a hole in the wall and ignited polyurethane foam surrounding some cables. He tried to beat out the smouldering foam with a flashlight, but succeeded only in burning the flashlight lens. He then tried to smother the fire with rags, but that didn't work. Somebody brought him a carbon dioxide fire extinguisher, but the CO₂ blew straight through the hole without extinguishing the fire. Fanned by the air rushing through the hole, the fire began to get further back into the wall. He tried firing off two dry chemical fire extinguishers, but without success, and about 15 minutes after the fire started, the evacuation alarm sounded in the cable spreading room where he was working.

The cable spreading room evacuated in preparation for tripping the permanent carbon dioxide extinguishing system. In the time, another electrician rang the alarm in a roundabout way (the fire alarm telephone number listed in some plant manuals, so there was sor

and the reactor operators in the control room were notified of the fire. By that time, the burning had travelled through the hole in the wall into the reactor room, where several cable coatings had caught fire. Firefighting efforts there were hampered by the fact that the burning cables were about 40 feet above floor level. The local fire department was notified at 1309 and fire trucks arrived at 1330 from Athens, about 10 miles from the plant.

Among the alarming features of the firefighting effort noted by NRC investigators were the following:

- The carbon dioxide system initially failed to work because the power had been shut off and metal plates had been placed over the manual cranks to guard against accidents during construction work.
- Smoke in the reactor building was so dense that the room was evacuated at about 1300, and "there appears to have been no central organised direction of the fire fighting efforts in this area until approximately 4.30 p.m."
- Soon after he arrived at the plant, the Athens fire chief recommended spraying the fire with water, but the plant supervisor refused to allow use of water because of the danger of electrical shorts and injury to the fire fighters. He eventually sanctioned the use of water at about 1900 and the fire was extinguished within 15 minutes. It was formally declared to be out by 1945. By that time, the fire had damaged cables about 40 feet into the reactor room and a few feet inside the cable spreading room.

In contrast to the firefighting efforts, attempts to shut down the reactors were more successful, but no less dramatic. Because some key equipment had been disabled by the fire, the reactor operators were forced to use what the NRC coyly describes as "unconventional" methods to shut one of the reactors down and to avoid a potentially serious situation.

The reactors are controlled in a common control room immediately above the cable spreading room. The operator in charge of the Unit 1 reactor started noticing strange behaviour of various indicators on his control panel about five minutes after he had been notified of the fire. He was getting erroneous indications that pumps were running, some pumps cut in automatically, power output from the reactor began to drop, and several lights on the control board began glowing abnormally bright and then getting dim or going out. At 1251, the operator decided to shut the reactor down by inserting the control rods into the core, thereby cutting off the chain reaction (in operator's parlance, he manually 'scrammed' the reactor).

That successfully halted the nuclear

reaction, but the problems then began. Because radioactivity decay of fission products in reactor fuel continues to heat the core long after the chain reaction is shut off, cooling water must be circulated through the core for many hours afterwards. Unless adequate cooling is provided, the reactor core could melt, burn its way through the concrete and steel floor of the pressure vessel and release large quantities of radioactivity into the environment. Because means of supplying cooling water to the reactor were knocked out by fire, the operator in charge of Unit 1 had to improvise.

Among the most important systems which were disabled was the emergency core cooling system (ECCS), a device which is supposed to flood the core with water if a pipe ruptures and the main cooling water is lost. The ECCS is used routinely during reactor shut-down operations.

According to the NRC report, and a description of the event provided to the Joint Committee on Atomic Energy last month by Donald F. Knuth, Director of the NRC's Office of Inspection and enforcement, the following sequence of events took place after the reactor was manually scrambled.

The water level surrounding the core dropped as a result of reduced boiling when the chain reaction was shut off. Consequently, pumps designed to inject water at high pressure into the reactor vessel increased their flow rate to keep the core covered with water. Soon after those pumps began running, however, a steam valve closed and disabled them. At that stage, the NRC report states, there was the following dangerous situation:

"The reactor coolant system had one remaining source of high pressure water, the control rod drive (CRD) system. Operator M (who was in control of Unit 1 operations) increased the CRD pump output to its maximum. Although the control room instrument has a maximum scale reading of 100 gallons per minute, Operator M stated that he knew that the CRD pump was pumping greater than 100 g.p.m. but he does not know how much was being pumped. Operator M advised the investigators that he did not think that starting the spare CRD pump would have resulted in significantly greater injection flow, and he recalls that the spare CRD pump was not always operable during the shut-down".

In other words, there was only one reliable means of injecting high pressure water to the reactor, and that was being strained to its limit to maintain the coolant level around the core.

It soon became apparent that the SRD pump system couldn't maintain

water level. It was therefore decided to open relief valves to reduce pressure in the reactor so that low pressure pumps could be used to supply cooling water. That tricky operation was carried out at about 1330, and the low pressure pumps provided sufficient water until about 1830, when control over the relief valves was lost and they slammed shut. Pressure in the reactor began to build up, and the low pressure pumps were rendered inoperable.

The operator was then forced to switch back to the CRD system to supply high pressure water. That arrangement provided sufficient coolant until about 2150, when control over the pressure relief valves was restored and the reactor was again depressurised. About 13 hours after the fire began, temporary repairs were completed to allow the reactor to remain at low pressure, and at about 0430, normal means of cooling the core were restored. By a series of extraordinary procedures, which have won praise from the NRC investigators, the reactor operators were therefore able to shut the plant down safely. But the incident nevertheless raises some very tricky questions.

Aside from the astonishing comedy of errors which led to the fire and caused it to burn for nearly 7 hours when it could have been extinguished with water in a few minutes, inadequacy in the design of the Browns Ferry plant was highlighted by the accident: a single fire, in a relatively small area of the plant disabled several key safety systems. Could a similar fire at another plant also have such potentially disastrous consequences?

Investigators from the NRC are now looking into the implications of the fire for other plants. According to Dr S. H. Hanauer, who is directing the investigation, important cables in plants built since Browns Ferry are more widely separated so that fewer key systems would be disabled by one fire. But "further improvements may be prudent in the light of the Browns Ferry lessons", Hanauer suggested, and he added that "some rerouting of cables may be necessary in some existing plants". Nevertheless, Hanauer stated that "based on our evaluation of the incident, we believe that even if a fire such as the one at Browns Ferry occurred in another existing plant, the most probable outcome would still be with no adverse effects on the public health and safety". The investigation, Hanauer said, "has not shown that present power plants are unsafe".

But the Browns Ferry fire had the makings of a very serious nuclear accident, and it has already provided plenty of ammunition for nuclear critics to shoot at the nuclear industry's otherwise good safety record. □

THE deficit in this year's grain harvest can hardly have come at a more embarrassing time for the Soviet planners. Just when internal propaganda is aimed at high output and increased productivity, at greeting the forthcoming Twenty-fifth Party Congress in a spirit of "socialist emulation" and celebrating the fortieth anniversary of "Stakhanovite" work, the Soviet government has had to admit, at least at the international level, that in spite of an ever-expanding programme of the mechanisation and electrification of agriculture, the harvest has once again failed to meet the basic requirement of feeding the Soviet population.

Scanning the Soviet press, it is difficult at first glance to find evidence of the deficit. On October 3, when the Soviet government was already seeking abroad the grain which it had failed to produce at home, *Radyans'ka Ukrayina* reprinted a speech of V. V. Shcherbyts'kyy, First Secretary of the Communist Party of Ukraine, which claimed: "Twenty regions have already fulfilled the agricultural plan [for grain delivery] ... and nine regions have overfulfilled the firm plan." Similar news has peppered the Soviet press throughout August and September. Collective farms, state farms, districts and regions have been reported, one after another, as fulfilling the plan.

Yet when one reads more carefully, a somewhat different picture emerges. Immediately before announcing his apparently promising picture of overfulfilled plans, Shcherbyts'kyy had admitted that hopes for a good harvest had fallen victim to "extremely unfavourable weather conditions", but that nevertheless, the total yield would be "considerably higher than in 1972" (cold comfort, since 1972 was the year when crop failures resulted in considerable food shortages). Later in the same speech, he mentioned the importance of guaranteeing adequate supplies of animal feed—implying that this year's reserves were dangerously low, if not actually insufficient. It may well prove necessary to slaughter off a considerable number of cattle on the collective cattle-rearing farms.

It should also be remembered that even if grain production quotas have been fulfilled, in the sense that the requisite amount has been delivered to the official collecting points, a bad harvest may mean that a collective farm is left with no surplus to be shared out for the support of its members and their domestic livestock for the coming year, causing the farm members to become, temporarily, consumers rather than producers, and necessitating a further depletion of

the country's livestock. Shcherbyts'kyy's speech refers, of course, only to Ukraine, but reports from the other grain areas of the Soviet Union, notably Kazakhstan and western Siberia, paint a similar picture.

The emphasis on delivery to the state of the prescribed quotas is fundamental to Soviet agricultural policy. In the early days of collectivisation, this led to considerable wastages. Forbidden by Draconian legislation to reap any grain for their personal use before the plans were fulfilled, peasants had to let their horses and oxen starve for lack of feed-

Alien corn?

from Vera Rich

stuffs, and so had no beasts left to draw the reapers when harvest could officially commence. Although such a situation would hardly be likely today, the precarious balance of Soviet agriculture, officially based on collective or state farms, but in reality deriving a considerable proportion of its vegetables and other small scale products from the personal allotments which the farmers cultivate in their free time is still liable to break down under any unusual conditions. Press announcements that peasants have been stealing "collective" grain to feed to their "private" cow or chickens can hardly account for deficits of millions of tonnes, but do, in some way, symbolise the whole problem of Soviet agriculture. The collective/state farm system simply cannot meet the demands made on it. At the popular level, the solution is that proposed by the Twenty-Fourth Party Congress: more mechanisation, more extensive use of fertilisers. The theoretical journals, however, show a deeper concern.

Last year, writing in *Planovoe khozyaistvo* (No. 3, 1974) the Chairman of the State Planning Commission noted a recent falling off of theoretical and methodological work on balancing the economy. Several follow-up articles in the same journal show considerable concern with the balance of the agricultural and industrial sectors. Similarly, writing in *Voprosy ekonomiki* (No. 8, 1975), M. Bronshtein, Corresponding Member of the Academy of Sciences of the Estonian SSR made an extensive analysis of the "Economic and Social Problems of the Industrialisation of Agriculture". The word "industrialisation" is significant here—Soviet planners see agriculture as being essentially one more branch of industrial production, amenable to the same system of fulfilment and overfulfilment of plans. The present trend towards

specialisation and concentration of different branches of the "industry" in different areas often, however, takes too little account of the fact that agriculture is what Bronshtein calls a "biomechanical system". His article is tentative and theoretical, but it does reflect a certain awareness that agriculture has its special problems which will not necessarily respond to an industrial-type treatment.

Although such discussions do take place at the theoretical level, however, Party policy demands the acceptance of the fundamental superiority of the Soviet agricultural system. On October 7, the Deputy Minister of Agriculture, A. A. Gol'tsov made a statement in passionate defence of the system. Although the final figures are not yet in, he said, an "average" harvest is expected. The Soviet Union is one of the world's leading grain producers. Grain yields are well up to international standards. Export and import of grain is "normal practice" in international trade. Grain purchases from the USA are intended for the Soviet Far East, to obviate the long haul across the Trans-Siberian railway. The Soviet Union not only imports grain but also supplies it to other countries. Protesting far too much, he then drew attention to the "steep growth" in agricultural production which has taken place since the 2.3 million tractors and 600,000 combines of today replaced the 17 million wooden harrows of Tsarist times. He stressed the great social benefits that collective farm life has brought to the peasants. Unfortunately, his argument breaks down on one vital fact—Tsarist Russia was a major grain exporter, the "breadbasket of Europe."

Four days later, addressing a rally of collective farmers, the Minister of Agriculture, Dmitrii Polyanskii, spoke far more realistically, admitting that "unusual weather conditions" would inevitably be reflected in the general level of agriculture as a whole. It is one thing, however, to admit a sudden emergency, but another to suggest that the system itself might be at fault. For this reason, although in the present atmosphere of detente the Soviet government is entering into all manner of trade agreements with Western and Third World countries, even in such sensitive areas as oil production or nuclear fuels, there has so far been considerable reluctance to make a firm agreement on grain purchases. Present negotiations, although described as being in a "very delicate" state, seem, however, to be moving towards the concept of a regular sale agreement. The stabilisation of the grain market thus produced of long term benefit to both

international news

Sakharov and the cause of peace

from Vera Rich, London

THE announcement last week of the award of the 1975 Nobel Peace Prize to Academician Andrei Sakharov coincided neatly with the 250th anniversary celebrations of the Soviet Academy of Sciences. This event, however, was not claimed by the academy as a culminating point of the celebrations, a tribute, as it were, to the efforts of Soviet science in the cause of peace. For the very activities which have won Sakharov the Peace Prize have brought him into disfavour with the Soviet establishment, and although he still retains his status as an academician, he does so on sufferance.

Sakharov's involvement in problems of peace and human rights has arisen as a direct corollary of his scientific career. After defending his PhD thesis (on cosmic-ray theory) in 1947, and after publishing only three scientific papers he disappeared from the world scientific scene into a top-secret establishment working on the development of the H bomb. At this stage in his career, Sakharov was convinced that a balance of power was the only sure guarantee of world peace and, "carried away by the immensity of the task", he contributed several key ideas to the research, although he has always eschewed the title of "father of the Russian H bomb" conferred on him by Western journalists, stating that the project was essentially one of "collective invention".

At the same time, together with Igor Tamm, he began working upon non-military aspects of thermonuclear reactions and plasma physics. It was for his work on the bomb, however, that he received (secretly) a Stalin Prize, three Orders of Socialist Labour, and a salary of 2,000 roubles a month, with numerous fringe benefits, such as access to restricted consumer goods, a chauffeur-driven car and a round-the-clock bodyguard. His election, in 1953, as a full member of the Academy of Sciences, without passing through the intermediate stage of corresponding member, was likewise a result of the military aspect of his work.

During the 1950s, however, Sakha-



Sakharov and his wife, Yelena Bonner: academician on sufferance.

rov's attitude to the nuclear arsenal began to change. He became increasingly concerned with the genetic and pollution effects of nuclear fallout, and also with the tremendous consumption of resources and manpower involved in making bombs. From 1958 onwards, he campaigned consistently against nuclear tests—earning from Khrushchev the comment that Sakharov was a good scientist, but should leave politics to the politicians. This, however, Sakharov was not prepared to do, and the campaigns continued. Finally he was able to urge the acceptance of a test-ban treaty covering the atmosphere, space and the ocean.

In 1964, Sakharov became involved in the great debate of the Academy of Sciences and the Academy of Agricultural Sciences which, after a quarter of a century of Lysenkoism, led to the reinstatement of genetics as a respectable branch of Soviet science. This event, apparently only an internal matter of academic policy, was to have great psychological significance for Sakharov. By now he was experiencing a complete revulsion from the whole concept of nuclear weaponry. He was increasingly disturbed by the ethical problems engendered by the bomb and by the often cynical attitude of politicians towards such problems. At this point, as a result of his participation in the Lysenko debate, he became acquainted with the biologist Zhores Medvedev, and through him with his

twin brother, the historian Roy. The Medvedev brothers introduced Sakharov to *samizdat*, the growing body of clandestine, self-published typewritten literature, which circulated among the growing circle of those who would later be called dissidents. In 1968, Sakharov made his own contribution to *samizdat* literature with a monograph entitled *Progress, Coexistence and Intellectual Freedom*.

This manuscript circulated widely in *samizdat* and inevitably reached the West, where it received considerable publicity. The Soviet government accordingly revoked Sakharov's security clearance, and it was almost a year before he could find another post—as a "senior researcher" at the Lebedev Physics Institute, the lowest grade of post in which an academician can legally be employed. Two years later, Sakharov published a second "manifesto", which was also signed by Roy Medvedev and a physicist, Valentin Turchin. This took the form of an open letter to the Soviet leaders, claiming that the major internal problems of the Soviet Union, the slowing down of the economy and a failure to meet the challenge of the "second industrial revolution" of the computer age could only be met by increased democratisation.

From now on, Sakharov was to be increasingly concerned with the problems of the individual. In November 1970, he formed, with Andrei Tver-

dokhlebov and Valerii Chalidze, a Human Rights Committee, maintaining that in doing so he was acting within the guarantees of the 1936 Soviet Constitution. Since then he has campaigned unceasingly for the release of dissidents illegally confined in mental institutions, for the rights of Jews to emigrate to Israel, for the abolition of the death penalty, for the rights of minority communities within the Soviet Union, for the amnesty of political detainees. In doing so, he has brought down on himself considerable pressure from the authorities and open attacks in the Soviet press which, in 1973, evoked fears that a show trial might be imminent.

His large personal fortune, amassed during his work on the H bomb, he donated, some years ago, to the Soviet government's Cancer Research fund, and although retaining his status as an academician, the material benefits normally accruing to such a position have steadily vanished. Furthermore, his scientific career has been interrupted by the "inner unrest" and by the increasing demands of all those who have come to regard him as a kind of ombudsman for the dissidents. He admits that he has "not been productive" of recent years, and on occasion has suggested that his most fertile years are behind him. Those who have had occasion to speak with him on scientific topics (mostly young refuseniks and others who find it difficult to continue their scientific career under the prevailing conditions) maintain, however, that his insight and scientific intuition is as active as ever and that a conversation with Sakharov can throw a whole new light on their work.

The award of the Nobel Peace Prize to Sakharov may be seen by many to contain a certain paradox. His early involvement in the H-bomb programme and his growing involvement with internal abuses in the Soviet Union rather than with the broad issues of peace between nations—these may seem strange qualifications for a Peace Prize winner. But it was in his involvement with thermonuclear weapons that Sakharov first came face to face with the ethical problems involved in their use; seeing peace and detente as the only alternative to a nuclear holocaust, and believing that the desire for peace is shared by the majority of mankind, he believes that the only sure guarantee of peace is that of a democratically based society, in which governments will permit a free discussion of all issues which concern the community and which, in the words of the Nobel citation "the inviolable rights of man . . . serve as the only sure foundation for a genuine and long-lasting system of international cooperation."

THE business of exporting nuclear technology to countries which are not signatories to the Nuclear Non-proliferation Treaty (NPT) has come in for serious criticism after the 'leaking' of secret documents implicating West Germany in the development of a South African programme capable of supplying nuclear weaponry. In Bonn, members of Chancellor Schmidt's government have spent the past few days issuing denials of involvement, denials of denials and even (from State Secretary Klaus Boelling) a 'correction' of his denial to make it an affirmation that Kraftwerksunion (KWU) had indeed asked for an official guarantee to export nuclear reactors to South Africa.

The row has already led to the resignation of Lieutenant General Gunther Rall, the West German representative on NATO's military committee, who was said to have visited South Africa secretly and under a false name, as a guest and adviser of the South African Defence Department. It has also led to a general reassessment of a policy under which Bonn has agreed, in spite of US opposition, to supply to Brazil a package deal involving a complete nuclear fuel cycle which would allow, if required, the manufacture of weapons-grade material.

The debate has been escalated by the news that Egypt has asked Bonn to cooperate on its nuclear programme, and that South Africa is reported to be planning the sale of 14,000 tons of uranium oxide to Iran in a deal which would give Iran a part share in a large uranium enrichment plant, capable of producing fissionable material and based in South Africa.

One of the most awkward problems in controlling the spread of nuclear weapons is the establishment of a mechanism to ensure that equipment and material supplied for civilian use is not actually converted to a military end. The West German-Brazilian deal brought the problem sharply to the attention of the countries capable of managing such sales (the USA, the USSR, France, West Germany, Japan and Britain) and they are reported to be having confidential talks to draw up a set of rules to govern nuclear exports. Whatever the outcome of the talks (and it is said that a set of safeguards is likely to be agreed by the end of the year) it looks as though the German-South African deal will go ahead, in spite of a rather vague undertaking from Bonn that the political implications as well as the economic will be considered before it agrees to issue a licence to KWU to build reactors for South Africa.

But even if the sale does go ahead, its accomplishment will be attended by a good deal of embarrassment for both the West German and South African governments. And the laying bare of the coy, undercover exchanges by which the two countries corresponded has been a thoroughly successful public relations exercise on the part of the African National Congress (ANC), which was responsible for the exposé.

Documentation for the deal, including the secret telegram which led to the downfall of Lieutenant General Rall, was among matériel believed to have been stolen by a spy from the South African Embassy in Cologne. It was published by the ANC in an account entitled "The nuclear conspiracy: FRG [Federal Republic of Germany] collaborates to strengthen apartheid".

According to the ANC, a uranium enrichment plant to be built in South Africa next year has been developed with the assistance of the state-owned Gesellschaft für Kernforschung (GFK) and the state-controlled company STEAG, with the agreement and active participation of the Bonn government. The prototype plant, it is estimated, will cost \$1,400 million to install, and an ancillary power station costing a further \$800 million will have to be constructed in order to provide energy for the process.

After making comparisons with the costs of conventional fuel sources, the ANC argues that a uranium enrichment plant in South Africa cannot be justified economically on energy grounds, and can only be explained in political and military terms. As Pretoria has refused to sign the NPT, the ANC claims, it will be able to use the enrichment plant "to produce uncontrolled material for nuclear weapons. The threat of proliferation could be used to deter embargoes and sanctions, while the threat to use nuclear weapons could be used to extend apartheid's hegemony on the continent of Africa. The use of nuclear weapons cannot be precluded as a desperate measure to preserve the apartheid state."

The suggestion is not simply paranoia on the part of a struggling black nationalist group. The South African state-owned broadcasting corporation reported only last week that "more and more countries are acquiring nuclear power stations, and with them the automatic capability of making nuclear weapons", and many of the country's leaders are committed to the development of a nuclear arms capacity as an integral part of a civil nuclear programme. □

THE European Science Foundation (ESF) last week marked its first year of existence with the publication of its first annual report and with a simultaneous venture into the field of public relations. Although it has clearly decided that it is now ready to present itself to the scientific community, the foundation did not have much in the way of positive achievement to report, other than its readiness to tackle the tasks it has set itself. Sir Brian Flowers, the foundation's first president, was, however, able, in part at least, to fend off a good deal of scepticism about the organisation's future effectiveness.

Sir Brian made clear last week that the sole aim of the foundation is to further international cooperation in new scientific schemes. He admitted, however, that little had been done during the past year apart from "organising the organisation", though one or two definite moves have already been made towards coordinating research projects. These include the incorporation into the foundation of the pre-existing European Medical Research Council (EMRC) and Western European Science Research Council (ESRC) and the setting up of an *ad hoc* Committee for Space Science and a Standing Committee on Astronomy. More specifically, the foundation has managed to implement international research projects in fields as widely scattered as genetic manipulation, particle physics, and the preservation of mediaeval stained glass.

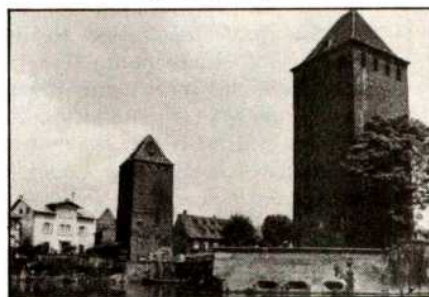
But Sir Brian denied that the foundation will ever be called upon to fulfil any role other than overseeing cooperation in pure research and preventing unnecessary duplication, adding that he saw no need for the ESF to become involved with the "interface between politics and science". From that relatively shaky standpoint it was easy for him to defend the ESF against sceptics expecting an easy time at the expense of an organisation operating on a budget of £200,000 with a full time staff of six based in Strasbourg. Nonetheless, the foundation still has to weather its first storm, and though its board may insist that the ESF can be effective in ensuring that there is no repetition of the situation whereby Britain, France and Germany all expend individual effort on the construction of broadly similar particle accelerators, it remains to be seen what weight the foundation carries when it comes to national chauvinism. At the moment there is optimism that the foundation will be able to influence participant governments through its member organisations—Britain's include the five research councils, the British Academy and the Royal Society—though what would happen, in the absence of finan-

cial control by the foundation, should these organisations themselves revert to nationalistic stances has not apparently been considered by the board.

All the same, Sir Brian's resolution and view of himself as first and foremost a European must count for something, and he instils an undeniable air of confidence throughout the foundation. He insists that the ESF should be run with a human touch, perhaps acceding to the point often made that it was established to pre-empt the bureaucracy of the Brussels administra-

Strasbourg diary

by Allan Piper



tion. Though not in as many words, what he apparently advocates is a return to grass roots policy-making, but within a pan-European framework. In a rare display of emotion last week, he expressed horror at the notion that the foundation should become nothing more than a central computerised clearing house of research data, preferring instead to envisage the setting up of "a little piece of Europe" for the scientific community.

Whether or not that small international community can expect to grow much larger in the near future is on the surface as indeterminate as many other aspects of the foundation. Though the admission to the foundation last Wednesday of the Greek National Hellenic Research Foundation brought the number of member organisations to 43, representing 16 countries, Sir Brian did not evince much enthusiasm for the suggestion that countries from the eastern bloc be admitted. Yugoslavia has, in fact, been a member since the founding of the ESF but Sir Brian feels that, though approaches from other communist countries would not be discouraged, the foundation for its part would be better off spending the next five years or so consolidating its position and will not make any overtures in that direction.

For the more immediate future, the Executive Council of the ESF has just passed fresh proposals recommending research projects involving, among other things, the general place of biology in space research, patterns of

human migration within Europe, the difference between existing European legal systems, and methods of teaching advanced mathematics. That selection identifies the eclectic scope covered by foundation, and illustrates the point made by everybody connected with its running that the word "science" should be interpreted in a broad European sense of knowledge or learning. Whether or not such an essentially academic approach will be able to survive in the latter half of the twentieth century only time will tell; in the interim, it is as well to share the optimism of the ESF representative who feels that when heads need knocking together, as they surely will, Sir Brian Flowers is the right person to do it.

● Out on the eastern edge of the city, in a solid concrete building unflatteringly reflective of a more substantial international role, the Parliamentary Assembly of the Council of Europe (CE) was last week convened for a nine-day plenary session. Ample evidence there of the political-scientific interface mentioned by Sir Brian Flowers. Essentially a political organisation the council has set up a European Joint Committee of Scientific Cooperation (EJCSC) comprising a mixture of scientists and parliamentarians engaged in the running of research groups and working parties. One obvious advantage which the council has over the ESF lies in the political muscle it has developed over 26 years of existence; thus, any findings coming out of the EJCSC may eventually come up for consideration by the council's Committee of Ministers, so that pressing issues of practical concern stand some chance of being satisfactorily resolved at an international level. During last week's session, for instance, the issue of pollution of the Rhineland water table came up for discussion. The problem is that the water table, lying below the surface, provides 80% of the water reserves for an area that covers parts of France, Switzerland and Germany, so that both the consumption and the pollution of the water transcend national boundaries. In the past there has been little attempt at international control but by bringing the issue to the attention of the CE a coordinated approach should be ensured over the coming months. It may seem invidious to compare the workings of a small, newly formed organisation expressly unconcerned with the problems of technology, with a well established political body working on a multimillion pound budget, but the comparison does illustrate that, whatever else the ESF may aver, politics and science are becoming ever more inseparable.

● There was an unmistakable air of satisfaction during the session over the

THE Natural Resources Defense Council (NRDC), which has long been a prominent thorn in the flesh of the nuclear industry, has now launched a drive for stricter radiation standards in the United States. In petitions filed during the past two weeks with the Nuclear Regulatory Commission (NRC) and the Environmental Protection Agency, NRDC staff scientists Arthur Tamplin and Thomas Cochran argue that workers exposed to radiation at the present maximum permissible levels run an unacceptable risk of dying from cancer and of having children with serious genetic defects. Consequently, they argue that the permitted exposure levels, at least for younger workers, should be reduced by about a factor of 10.

On the basis of several reports and studies published in the past few years (particularly a 1969 report by the International Commission on Radiological Protection and a report on the biological effects of ionising radiation (BEIR) published in 1972 by the National Academy of Sciences), Tamplin and Cochran have calculated that between 2 and 6% of all radiation workers exposed to whole-body radiation at maximum permissible levels for their working lives could die from radiation-induced cancer.

Though they point out that few radiation workers are in fact exposed to the maximum permissible levels of radiation, Cochran and Tamplin suggest that such a risk is "excessive" and that the standard is an "inappropriate guideline" for the nuclear industry.

Similarly, the petition contains calculations suggesting that workers in the nuclear industry who are exposed to maximum permitted levels of whole-body radiation would stand between a 1 in 10 and 1 in 300 chance of having children with serious radiation-induced genetic defects.

The present maximum allowable whole-body dose of radiation is 5 rem a year, and no more than 3 rem in one calendar quarter. Cochran and Tamplin argue that the standard should be replaced by a requirement that workers under the age of 45 should receive no more than 0.5 rem a year to the whole body, and not more than 0.3 a quarter.

Older workers would be allowed higher radiation doses—up to a maximum of 3 rem a quarter, however.

A two-tier exposure level, Cochran and Tamplin argue, would offer the greatest protection to those most susceptible to radiation injury, such as pregnant or fertile women, while not at the same time applying the strictest standards to other parts of the workforce. "Hence, the proposed changes

Washington seen

by Colin Norman

should not place a large burden on the industry", they argue.

If the NRDC's proposals were adopted, the greatest impact would be felt in fuel-reprocessing and plutonium-fabricating plants, but no such facilities are yet in commercial operation. The immediate effect of the petition, however, is likely to be to reopen the long standing debate about the consequences of long term exposure to low levels of ionising radiation.

● Following recent revelations that the Central Intelligence Agency (CIA) and the Department of Defense have been carrying out ethically bankrupt research on people—such as feeding psychotropic drugs to soldiers without their knowledge or consent—Senator Edward Kennedy has proposed a bill to establish a Presidential commission to oversee all federally supported clinical studies.

The bill would essentially transform a panel which was established by Congress last year to draft regulations governing human experimentation supported by the Department of Health, Education and Welfare, into a permanent body with broad responsibilities. Called the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, the panel has already issued recommendations for research on foetuses, and it is now trying to draft guidelines for research on prisoners, children and the mentally ill. It is expected to complete its work early next year.

Kennedy said last week that the abuses perpetrated by the CIA and the Department of Defense "underscore

the need to expand the national commission's jurisdiction, and to expand it now". His bill would add the Secretary of Defense, the Secretary of Health, Education and Welfare, the Director of the CIA, four Senators and four Representatives to the panel's 11 existing members. It would also establish the commission as a permanent advisory body with responsibility to make public recommendations on clinical studies sponsored by all government departments.

Since Kennedy is the chairman of the Senate Health subcommittee, to which the bill was referred, and since it is also backed by two senior republicans on the subcommittee—Jacob Javits and Richard Schweiker—it stands a very good chance of being passed by the Senate.

● The latest in a long line of statements by prominent scientists warning about the dangers of nuclear power was made public with considerable fanfare last week. It is particularly noteworthy for two reasons. First, it resulted from a study sponsored by the National Council of Churches—a body with considerable influence. And, second, it was supported by a galaxy of scientific stars, including 14 Nobel Prizewinners.

Called "The Plutonium Economy: A Statement of Concern", it warned about the dangers of allowing plutonium to be extracted from spent reactor fuel rods and recycled as a nuclear fuel, and it spoke out against the development of the fast breeder reactor. "We believe that the proposed 'plutonium economy' is morally indefensible and technically objectionable", the statement said.

Drafted by a committee chaired by Rene Dubos and Margaret Mead, and supported by a lengthy background report on the dangers of plutonium, the statement was requested by the National Council of Churches last year. After remarking on the dangers of using plutonium, the report concludes that "all who believe that technology should serve human values should join in opposing the plutonium economy and in seeking to divert into more constructive channels the vast resources being devoted to nuclear power."

council's role in the setting up of the recently formed European Space Agency (ESA). As long ago as 1960 the council was calling for one European agency to handle the building of both launchers and satellites, and the amalgamation of ELDO and ESRO last May was regarded by most delegates as the results of persistent lobbying by the council. The feeling of achievement was celebrated last week by the assembly when it unanimously

agreed a draft recommendation that member states of ESA meet at least once a year at ministerial level. The feeling is that ministerial meetings will provide the agency with an impetus which might otherwise be lacking.

American confidence in the agency has been demonstrated by a request for the ESA to cooperate in NASA's post-Apollo programme: a space laboratory is to be built by European industry under the guidance of the agency.

Apart from scientific and technological benefits, a fully independent ESA could also provide industry and employment—there is even a whisper that the agency ought to become outwardly commercial and seek to establish new markets within the Third World. One delegate, perhaps mindful of the approach of 1984, even expressed relief that with a fully independent space industry Europe will be in the running for worldwide communications control.

correspondence

First-year ecology

SIR,—Although I sympathise with many of the sentiments expressed by Dowdeswell and Potter (October 2) it seems to be that some of their difficulties could be overcome by giving up the search for "natural" habitats (which hardly exist in Britain) and instead concentrating on the immediate surroundings. Take, for example, the ordinary suburban garden. Its high diversity of plants supports an immense variety of animals (most of them insects) and an array of ecological situations reminiscent of tropical rain forest. Virtually all the principles necessary for a first-year ecology course can be demonstrated and investigated in a garden. There is no shortage of material and an imaginative ecologist is soon able to find examples of what he wants.

There are populations of aphids, ladybirds, earthworms, and woodlice for practical work in population ecology. There are intricate food webs centred around every plant and tree. The onion bulb fly, the two-spot ladybird, the peppered moth, and a host of others, can be used as examples of the gene as an element of continuity in populations. I think that the trouble is that rather little is known and published about the ecology of gardens. Research money, as we all know, has gone to the study of "natural" areas and farmland and gardens have been dismissed by some ecologists as biological deserts, which is a pity, for here is a vast expanding resource invaluable to teacher and conservationist alike. Indeed the fact that so little is known should be exploited in the development of a spirit of inquiry among first-year students. Finally, although I would not dispute the value of simulation experiments and visual aids I remain sufficiently old-fashioned to believe that direct contact with nature is better.

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Deterioration

SIR,—Whereas the quality of the scientific contributions to *Nature* remains uniformly of the highest calibre, I have noticed over a period of several years a steady deterioration in the quality of editorial contributions.

A recent example (August 21) is the

astonishing revelation that the old Chinese had discovered relativity, galaxies, perhaps pulsars and X-ray stars. Surely the author should re-read his classics to find out that, far from being 1,300 years behind, western thought was in fact several hundred years ahead of these Chinese speculations.

In Lucretius's *De Natura Rerum* (about 55 BC), for example, we find also ideas about empty space ("All nature as it is in itself consists of two things—bodies and the vacant space in which they are situated", Book I, 418) and about time and space ("It must not be claimed that anyone can sense time by itself apart from the movement of things", Book I, 460). All this was preached by Democritus in the fifth century BC, but many other examples of anticipations could be found in the writings of Greek philosophers.

I fear that the review article in question is indicative of a spirit of masochistic depreciation of science in general and Western culture in particular, which is regrettable in the offices of so prestigious a scientific journal. Many readers will also remember that ample space was given to an Indian journalist for a vicious attack against the USA and the WHO, and that only several months later did the editor feel obliged to shed some crocodile tears in an editorial more designed to whitewash himself than to put things right.

Yours faithfully,

Dr S. V. VAECK

Hofstade, Belgium

Collectors' Code

SIR,—In recent years there has been a good deal of comment on the remarkable zeal shown by some American bird and egg collectors, which led to much discussion at the XVI International Ornithological Congress in Canberra last year (*Nature*, 248, 543; 249, 793; *New Scientist*, 64, 734; 1974). The leading American learned society, the American Ornithologists' Union, set up an *ad hoc* committee under the eminent conservationist John Aldrich to consider the whole matter, and they have now produced a lengthy report.

After emphasising the number of birds killed by hunters and pest-control agencies and the need for scientists to have adequate freedom to

obtain specimens, with which few reasonable people would argue, it ends by advocating some relaxation of local controls, on which it is difficult to comment without more knowledge of the local situation, and proposing a "Code of ethics for collectors and capturers of birds" with which few can disagree and doubtless most will warmly welcome. Since you were at one time very helpful with this campaign, and since the code surely deserves careful study by members of many disciplines throughout the world it may be useful to reprint it:

- The privileges of a collecting or capturing permit shall be used only to obtain specimens for justifiable scientific or educational purposes.

- Collect or capture specimens only from those populations or species that can sustain the loss of individuals.

- Collect or capture only those specimens that are deemed necessary and that can be properly cared for or prepared

- Exercise the greatest care in recording accurately the maximum amount of relevant data for all specimens obtained.

- If live birds are collected, maintain them under humane conditions with high standards of health and sanitation.

- Collect with the aim of making available all relevant data obtained from specimens, either through publication or by giving access to the data.

- Abide by all stated regulations, including the use of authorised permits to collect, capture, import, export and trans-ship specimens.

- Notify the appropriate local authorities of plans to collect or capture birds in areas under their jurisdiction.

- Identify yourself and your purposes to those who may witness your collecting or capturing in order to inform them of the validity of your activities.

- Be as judicious and humane as possible in collecting and capturing activities, taking care to respect the rights, interests, and feelings of others.

- Regard the privilege to collect or capture birds as a trust in the pursuit of science; it should never be flaunted.

Yours faithfully,

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news and views

THE application of nuclear magnetic resonance (NMR) spectroscopy to biochemical systems is increasing at a prodigious rate, with the emphasis now clearly in the direction of enzyme-substrate interactions. As more and more sophisticated interpretations are brought to bear on indirect measurements, it becomes increasingly difficult for the people most interested in the biological implications of the experiments to follow the reasonings and assess the significance of the results. What, for example, does the fact that ion A is $x(\pm y)$ nm from atom B of substrate C bound to enzyme D tell us, in the absence of crystallographic data on the crystal structure? Those who have such reservations will surely welcome the trend exemplified by a research group from the National Institute of Medical Research in London. This group has been pursuing investigations into the enzyme dihydrofolate reductase, and its latest paper (page 564) concerns the direct observation by ^{31}P NMR spectroscopy of NADPH (a dinucleotide cofactor in the enzyme reaction) bound to this enzyme. The authors have been able to study some properties of the bound molecule directly, revealing some facts about the nature of the binding, and are clearly very close to being able to determine its conformation, well in advance of the determination of the enzyme structure by more conventional means.

Biological studies using ^{31}P NMR have been relatively few compared with those using proton NMR. This has been due largely to two factors. First, nearly all biological molecules contain hydrogen, but not all contain phosphorus.

NMR and enzyme binding

from G. E. Chapman

Second, the sensitivity of ^{31}P to NMR detection is about an order of magnitude less than that of protons, and hence correspondingly higher concentrations are needed. But ^{31}P has some distinct advantages over protons for NMR study. In particular the chemical shift range is greater, and linewidths are less than those of protons on the same molecules. This means that more fine structure may be observed in the ^{31}P NMR spectrum of a large molecule than in its proton NMR spectrum, and hence there is more potentially useful information to be extracted.

The earliest published papers in this branch of biological NMR dealt with nucleotides and related molecules, where the technique was used principally to investigate solution conformations in conjunction with proton NMR. Then work began to appear on phosphoproteins, nucleic acids and phospholipids, but it is probably fair to say that the triumph of observation outweighed any other scientific significance, at least in the earlier publications. A paper on ^{31}P NMR of tRNA fractions by Gueron (*FEBS Lett.*, **19**, 264; 1971) appeared to show the promise that conformational information could be derived from the chemical shift perturbations of the phosphodiester groups (in the same way that has been done for proteins by proton

NMR) but little has been heard since in this direction. The application of Fourier transform techniques to NMR caused a dramatic decrease in the concentrations of nuclei needed for observation. As a result of this, Richards and his coworkers at Oxford could achieve the remarkable feat of monitoring the changing levels of phosphorus-containing tissue metabolites in an intact muscle during tissue death, using ^{31}P NMR (*Nature*, **252**, 285; 1974). This highlights the potential value of NMR as a non-destructive assay technique. This type of experiment has been extended to the study of a developing embryo. There appears to be an additional bonus in that the chemical shift of the inorganic phosphate resonance makes possible an estimation of the intracellular pH without physiologically perturbing the system.

Evidence is accumulating that ^{31}P relaxation is dominated by chemical shift anisotropy at high magnetic fields. This apparently esoteric tidbit is not without significance to prospective researchers in the field: in essence it means that the resolution of chemically different phosphorus nuclei in a sample, far from being increased by going to higher magnetic fields will probably be decreased. Thus there is much scope for work at the field strengths attained by conventional magnets, rather than using the much higher field (and costlier) superconducting magnets.

It is to be expected that ^{31}P NMR will play an increasing role in the study of biological systems at the molecular level. □

BRIGHT meteors often leave a faint, persistent luminosity along the visual path after they have passed. For most meteors this luminous train lasts for a very short time, but for some it may be observable for seconds, minutes or even an hour. This persistent train is not formed along the whole path of the glowing meteor nucleus but only along that part of the path which falls within a specific height region of the atmosphere, usually between 85 and 90 km above the Earth. The colour of the train is green or yellow, fading to white as the train decays, the observed spectrum consisting of very few bright lines. Trains illuminated by sunlight, however, are red or orange, mainly due to the reflection of sunlight from the

Persistent meteor trains

from David W. Hughes

fine dust particles left behind the ablating meteoroid. Trains expand laterally at a rate of about 1.6 m s^{-1} , so a train which persists for an hour is about 11 km across at the end of this time. Train luminosity, I , decays according to the formula $I = (a + bt)^{-2}$ where a and b are constants and t is the time.

Many meteor trains appear double after a time, the train gradually becoming a tube of luminous matter which,

viewed from the side, appears like a double line of light. This effect may be caused by the dying out of the luminosity along the train axis or by the expanding edge of the train having a greater luminosity. If the luminosity was distributed evenly throughout the cylindrical train it would appear brightest in the centre when viewed from the side. During their lifetime the trains are blown about by the prevailing winds in the mesopause region of the atmosphere and in the first quarter of the twentieth century meteor train movement was the only indicator of the winds and turbulence in the upper atmosphere.

Meteor showers differ strikingly in the relative numbers of persistent

meteor trains produced. For example, about 50% of the meteors in the Perseid shower (which maximise around August 13 each year) leave trains, while only about 5% of the Geminids do so (maximum rate on December 14) Plavec (*Bull. astr. Insts Csl.*, 2, 19; 1949) concluded that the relative number of trains depended on the geocentric velocity of the meteoroids in the shower, this being 60 km s⁻¹ for the Perseids as opposed to 35 km s⁻¹ for the Geminids. Lindblad (*Meddn Lunds astr. Obs.*, Ser. 1. No 189; 1956) found that there was a direct linear relationship between the mean visual magnitude of the train and its duration, the brightest lasting longest. Also brighter meteors were more prone to have trains. Meteor magnitude, however, is not the only factor to be taken into account and often during shower observations bright meteors appearing in the same part of the sky and within a few seconds of each other differed radically in train-forming ability. Lindblad carried out radar and visual studies of the same meteor and found a clear tendency for radar echoes of exceptional duration to be associated with the presence of a visual train. In fact the train formation process is statistically more closely linked to the phenomena of a long duration echo than to that of a bright meteor—it is more dependent on the ionisation produced by the meteoroid than on the luminosity. In the magnitude range +2 to -1 the radar echo duration is twice as long for the train-forming meteor as for the non-train meteor.

The mechanism responsible for the persistent luminosity is still something of a mystery. In 1907 Trowbridge (*Astrophys. J.*, 26, 95) suggested that gas phosphorescence was the cause and compared the phenomena with the afterglow produced in a gas by the electrodeless ring discharge. Chapman (*The Aurora and Airglow*, edit. by Armstrong, E.B., and Dalgarno, A., 204, Pergamon, 1955) thought it unlikely that the source of energy emitted over so long a period could be the meteor itself, and suggested that the luminous energy was drawn from a pre-existing store in the atmosphere itself, such as the energy of dissociation of oxygen. It is also necessary to explain why some but not all meteors from the same stream and of the same brightness produce trains. Chapman suggested that meteoroids contain some catalyst that can draw on the atmospheric store of energy and convert it into light and that this catalyst is only present in sufficient quantities in a certain percentage of the meteoroids. Sodium, which as the normal airglow shows can glow through the night, was suggested as the most probable candidate: it would

transform atomic oxygen to molecular oxygen by converting the dissociation energy of O₂ into sodium light.

Baggaley in this issue of *Nature* (page 567) produces quantitative calculations of the energetics of this sodium process. The complete sequence of necessary chemical reactions is known together with their relative rate coefficients. Also the concentration of sodium in the incident meteoroid is known with fair confidence because meteoroids have been found by spectroscopic analysis of the emitted light to have the same elemental composition as carbonaceous chondrite meteorites. The persistent trains seem to be confined to the 85–90 km height region of the atmosphere and rocket borne mass spectrometers have yielded the chemical concentrations of O, O₂, O₃ and N₂ in this region.

Using all these observations Baggaley concludes that a meteor of magnitude about -10 (that is a tenth as bright as the full Moon) is required to introduce enough sodium to account for the observed luminosity. These very bright meteors are exceedingly rare—13 times rarer than the persistent meteor trains which last more than half an hour.

So it seems that the sodium process cannot provide sufficient luminosity and aeronomists must think again. Possibly the role of ionised species and the peculiar tube-like appearance of the train provide a clue. □

Information transfer in mammalian cells

from M. Bobrow and E. Solomon

Part of this article was inadvertently omitted last week. The earlier part described several situations in which genetic information, varying in amount from the whole nucleus to a small chromosomal fragment, can be transferred between different types of eukaryotic (and particularly mammalian) cells.

Transfer of genetic information by isolated mammalian DNA was claimed some time ago by Bendich, Borenfreund and Honda (*Informative Molecules in Biological Systems* (edit. by Ledoux), 81, North-Holland, 1971). Chinese hamster cells were incubated with mouse DNA and 1 in 10⁵–10⁶ cells subsequently appeared to react with an antiserum made to mouse cells. The alterations in these cells has not been further characterised. In a somewhat different situation, the technical difficulties in work of this sort are well demonstrated by the recent evidence (Kleinhofs *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, 72, 2748; 1975) that bacterial

contamination was responsible for early claims of the integration of isolated DNA into plant chromosomes. There is always the possibility that misleading results can be given by bacterial, mycoplasma, or viral contamination of the material used.

The same group (Bendich, Borenfreund, and Sternberg, *Science*, 183, 857; 1974) have recently investigated another potential system for transfer of genetic information. Mouse spermatozoa were coincubated with Chinese hamster fibroblasts and a proportion of these fibroblasts acquired the capacity to react with an antiserum to mouse embryos. Similar results have been obtained with rat sperm and Chinese hamster fibroblasts and were reported in *Nature* last week (Higgins *et al.*, *Nature*, 257, 488; 1975). Clones of cells from these experiments react with an antiserum to rat embryo, but have no detectable rat chromosomes on karyotyping. This situation is therefore somewhat similar to the various experiments described earlier, in that a genetic function appears to be transferred in the absence of any specifically identifiable chromosome from the donor. The HPRT enzyme transferred by way of isolated metaphase chromosomes has, however, been well characterised by a variety of independent techniques as being of donor species origin, whereas the information transferred by coincidence with spermatozoa has thus far been defined only by immunofluorescence with a relatively crude antiserum to an undefined antigen in murine embryonal tissues.

Spermatozoa are a cell type evolved specifically to the function of cell fusion and the transfer of genetic information; and yet (although it is difficult to document from the published literature) many workers have tried without success to use spermatozoa in cell fusion experiments of various sorts. The specific interest in transferring genetic information from sperm as opposed to any other cell source is that if the technique could be sufficiently refined it would open the way to direct analysis of meiotic products, give access to a genome which has not yet been modified by the processes of tissue differentiation, and it could conceivably throw some light on the natural process of fertilisation. The possible production of embryonal antigens, and their relationship to malignant change is the particular interest of the group presently investigating this system.

The experimental transfer of eukaryotic genetic information by nuclear transplantation or virus-mediated cell fusion has already been proven of tremendous value in several fields of biological research. Whether a similarly bright future awaits the recently de-

scribed techniques for transferring smaller amounts of genetic information is difficult to predict. If, with the development of appropriate selective systems, it should turn out to be regularly possible to introduce bits of chromosome containing one or a few specific genes into host cells, this could turn out to be of major significance.

Year of the ψ

from W. T. Toner

The Lepton-Photon Symposium was held at Stanford University on August 21–27.

It is appropriate that this year's Lepton-Photon Symposium was at Stanford where many of the discoveries which have revitalised high energy physics have been made. Pride of place went to new results from electron-positron annihilation, many of them obtained at the host laboratory.

The spectroscopy of the ψ , as it stood before the symposium, was reviewed in *News and Views* for September 11. Two new states were added at the meeting, to make a total of at least eight. R. Schwitters (SLAC, Stanford) reported data from the SLAC-Berkeley collaboration showing a fourth ψ -like particle of mass 4,400 MeV and width 50 MeV, produced directly in electron-positron annihilation. B. Wiik (DESY, Hamburg) and J. Heintze (Heidelberg) both presented evidence for the decay chain $\psi(3095) \rightarrow \gamma X(2800) \rightarrow \gamma\gamma\gamma$, and Wiik tentatively suggested (on the basis of two events) that X may also decay to proton-antiproton pairs. Spins and parities remain to be determined except for the two lightest ψ s (1^-), but the uncanny resemblance of the level structure to that of positronium (Mills *et al.*, *Phys. Rev. Lett.*, **34**, 1541; 1975) left few to argue against some form of charmonium interpretation (Appelquist *et al.*, *Phys. Rev. Lett.*, **34**, 365; 1975; Eichten *et al.*, *ibid.*, 369).

If 1975 is the year of the ψ , 1976 may well be the year of the lepton. Earlier this year M. Perl (SLAC) had reported on 24 anomalous events of the type $e^+ + e^- \rightarrow \mu^\pm + e^\mp +$ unobserved particles, of which only 5 could be background from conventional processes. G. Feldman (SLAC) discussed the extension of this analysis to cover the whole of the SLAC-Berkeley data, leading to a total of 86 events, of which no more than 22–30 could be from conventional processes. It is possible that these events are due to the production and weak decay of a pair of charmed mesons, but the most

popular explanation (as yet without proof) is that the reaction is $e^+ + e^- \rightarrow U^+ + U^-$ where U is a heavy lepton, a member of the series e, μ, \dots and decays to $\mu \bar{\nu}_\mu \nu_U \rightarrow \nu_U$ is the neutrino associated with U. The excitation curve suggests a mass of about 1.9 GeV for U.

The most apt comment in the 36 years of our attempts to understand the leptons, is Rabi's "Whoever ordered that?", of the muon. With a third member of the series one might begin to make progress. F. Gilman (SLAC) remarked that the U (if a lepton) has been found where charmed mesons were expected, just as the muon was found where Yukawa's pion was sought. He left unspoken the outrageous thought which occurred to more than one member of the audience: that there might be a connection between the masses of these leptons and hadrons—the pion is not much heavier than the muon, and the charmed mesons (when found) should have masses ~ 2 GeV. H. Harari (Weizmann Institute) presented an imaginative scheme linking leptons to hadrons in another way: the electron to the u and d quarks which are found in ordinary matter, the muon to the s and c quarks to be found in strange and charmed particles respectively, and the U to new t and b quarks, which would make up other families of particles yet to be discovered.

L. Lederman (Columbia University) discussed old and new data on the direct production of the old leptons (muons and electrons) in the strong interactions of protons, a process that should not take place to any appreciable extent, but does (*News and Views*, March 13). The plot seems to be thickening, with reports of enhanced production, relative to pions, at small transverse momentum, which rules out any explanation in terms of large transverse momentum phenomena, and also makes it difficult to explain the effect in terms of any very heavy particle decaying into lepton pairs. There is experimental confusion at low energies, with new data from a Pennsylvania-Stony Brook collaboration showing direct lepton production at energies as low as 10 GeV, in agreement with Leipuner *et al.* (*Phys. Rev. Lett.*, **34**, 103; 1975), but in disagreement with Winter (*Phys. Lett.*, **B57**, 479; 1975) and with Russian data reported to the symposium. The new discoveries at the electron-positron storage rings do not seem to be related to the high-energy direct lepton production in any obvious way, and if direct leptons are indeed produced at low energy, a relation between the two is extremely unlikely. There is no explanation for this phenomenon, nor even any estimate of its likely significance. As his conclusion,

Lederman showed a blank transparency.

The symposium would still have been stimulating without all this excitement. There was, for example, the strong evidence presented by Schwitters for the production of back-to-back jets of particles in electron-positron annihilation at 7.8 GeV, in beautiful agreement with the parton model; there was the report by W. Lee (Columbia University) of the first clear observation of the long sought decay of the ρ' (1600) into two pions; and a great deal more. There was also the 'monopole' event (Price *et al.*, *Phys. Rev. Lett.*, **35**, 487; 1975), which L. Alvarez (Berkeley) argued, most convincingly, was probably caused by the fragmentation of a platinum nucleus in the detector.

There was such euphoria about the new physics that several theorists—Gilman, Harari, C. H. Llewellyn-Smith (Oxford) and J. D. Bjorken (SLAC)—went out of their way to remind us that much of the interpretation rests on plausible assumptions, which remain to be proved. This will require a searching re-examination of the old physics as well as the new. \square

More headaches from star formation

from M. G. Edmunds

A RENEWED discussion of the interpretation of observational studies of the radio spectra of molecules in the Orion nebula will probably make star formation theorists reach for their aspirin bottles. The theory of star formation is complex and difficult; the refinements of competing physical processes that can be invoked are almost infinite, and yet even the most simplified models gobble up large amounts of computing time, only to leave the proponents arguing about numerical errors.

The basic model attempts to follow the self-gravitational collapse of a cloud of interstellar medium into a state where it is hot and dense enough for the start of nuclear energy generation and the birth of a star. Any observational constraints on the theories are invaluable since the conditions involved must range over many orders of magnitude in temperature and density as the formation proceeds. The presence of large amounts of finely-divided dust in the dense gas clouds which are the site of star formation means that radiation at optical wavelengths is obscured and the probing of the physical conditions can only be achieved with radio and infrared techniques. One of the happiest hunting grounds for the elusive observational data has been the region of the Orion nebula, which includes both the optically visible region of gas heated

by young hot stars, and a nearby extended region of high infrared luminosity believed to represent a nursery of stars in the process of formation.

Zuckerman and Palmer (*Astrophys. J. Lett.*, **199**, L35; 1975) consider the molecular lines in the radio spectra of the Orion infrared complex. They point out that the lines show a composite spectrum of a sharp spike superimposed on a broad plateau. The widths of the lines may be attributed to broadening by the Doppler effect as a result of gas motions; the spike implying velocities of about 4 km s^{-1} and the plateau implying much larger velocities of perhaps 30 km s^{-1} . While the low velocity region extends over a large region, the high velocity region appears much smaller and is centred on an area where the infrared emission is very high, including the sources known as the Becklin-Neugebauer object and the Kleinmann-Low infrared nebula. It therefore seems that the gas in a small region of Orion experiences much greater motions than the rest of the nebula. The most obvious interpretation is an increased velocity as a result of a conservation of energy and angular momentum during the collapse of part of the gas to form a protostar. But it is puzzling that inorganic molecules such as SiO, SO, H₂S are far more prominent in the high velocity region (compared with molecules containing carbon) than in the low velocity region. Since the

conditions required to excite the lines of these inorganic molecules are very similar to those required for the organic compounds, the implication is that the molecular composition of the gas in the Orion nebula is not homogeneous. Zuckerman and Palmer speculate that the elemental composition of the gas may not be homogeneous, and if this were the case then the consequences for the chemical composition of the forming stars would be fundamental. A less extreme view is that the conditions for molecule formation differ between the kinematically distinct regions, while the elemental abundances remain constant.

There is an alternative interpretation of the observations. Zuckerman and Palmer recall the suggestion that the Becklin-Neugebauer object represents an evolved F star whose radiation is very reddened by dust (Penston, Allen and Hyland, *Astrophys. J. Lett.*, **170**, L33; 1971). Indeed, the only other "plateau" molecular line source yet found is a carbon star whose molecular spectrum shows organic molecules formed from outflowing gas rich in carbon synthesised by nuclear reactions during the star's evolution. The Orion plateau source could therefore be explained as a region of gas ejected from a normal evolved oxygen-rich star in which molecules formed from oxygen dominate over those formed from carbon. There have been objections to the interpretation of the very strong

Becklin-Neugebauer object as a reddened evolved source, but if the above argument is correct there is justification for the view that at least some of the infrared sources in the Orion complex represent evolved stars rather than natal protostars.

The more depressing news is that Zuckerman and Palmer snatch away from theorists some observational determinations of the magnetic field in regions of star formation. They suggest that the existence of high velocity broadening effectively vitiates a recent determination of a very strong magnetic field by the Zeeman-effect broadening of SiO lines. They also point out that observational selection effects in OH line Zeeman-effect determinations, and uncertainties over what density of region these maser lines originate in, exclude such measurements as a guide to the general magnetic field within the nebula. They finally suggest that the measurement of polarisation of infrared emission induced by the alignment of grains in a magnetic field will not even give the field to an accuracy better than a few orders of magnitude. All this is very frustrating to model makers since, in a slightly ionised gas cloud, the magnetic field acts as an effective pressure and may be considerably enhanced as the cloud collapses. The strength of the field is thus an important parameter in determining the dynamics of the collapse. □

HALF the elements of the first transition series, namely manganese, iron, cobalt, copper and zinc have biochemical roles which are well documented and, in the main, thoroughly investigated. Of the remaining five elements of the series the investigation of three is increasing in popularity. Since 1911 (Henze, M., *Z. physiol. Chem.*, **72**, 494) vanadium has been known to occur in high concentration in the blood cells of ascidians—sea squirts—although neither its function nor its chemistry is yet understood. More recently both chromium and nickel have come under scrutiny for possible biological roles.

Chromium is now known to have an important function in nutrition in both man and animals, being involved in glucose metabolism (Merz, W., *Physiol. Rev.*, **49**, 163; 1969). Dietary studies have shown that induced chromium deficiency in rats and monkeys leads to impaired glucose tolerance, giving rise to a diabetes-like syndrome. This observation naturally led to studies of the effect of chromium on the action of insulin. Enhancement of the response of adipose tissue and mitochondria to insulin was observed with many chromium (III) species although

Biochemistry of transition metals

from A. J. Thomson

non-labile complexes such as [Cr(III)(ethylenediamine)]³⁺ were ineffective. In view of the recent detailed structural studies of insulin by Hodgkin and co-workers (*Proc. R. Soc.*, **B186**, 192; 1972) this problem invites further examination.

Nickel stood out among its transition metal neighbours as apparently being without biological effect. Sunderman and his colleagues have long sought one, concentrating at the outset on the toxicity of nickel carbonyl (*Ann. N.Y. Acad. Sci.*, **199**, 300; 1972). They have now demonstrated that nickel is maintained in the blood sera of man and experimental animals at levels between 2 and $12 \mu\text{g l}^{-1}$. The element is especially abundant in rabbit serum, facilitating isolation of a protein containing nickel, termed nickeloplasmin, which immunoelectrophoresis identified as an α_2 -macroglobulin. The protein has a high molecular weight ($>7 \times 10^5$) and contains 0.9 g atoms of

nickel per mol of protein. Now a group of Australian workers (Dixon, N. E., Gazzola, C., Blakeley, R. L., and Zerner, B., *J. Am. chem. Soc.*, **97**, 413; 1975) have advanced the claim that jack bean urease is a metallo-enzyme containing nickel. Although it was the first enzyme to be crystallised by Sumner in 1920 the metal content had escaped detection. This group has now succeeded because they had available a large quantity of highly purified enzyme. Atomic absorption and gravimetric analysis gives an estimate of 2.0 ± 0.3 g atoms of nickel per 105,000 g of enzyme. The electronic spectrum has been recorded and confirms the presence of the d-d transitions of the nickel (II) ion. Since the extinction coefficients of octahedral nickel (II) d-d transitions are very small, usually of the order of $10 \text{ mol}^{-1} \text{ cm}^2$, the detection of these bands requires long path lengths of highly concentrated material. For example, to detect the spectrum of nickel (II) in carboxypeptidase A, in which the naturally occurring zinc ion had been replaced by nickel, it was necessary to use 50-mm path length cells and $\sim 5 \times 10^{-4} \text{ M}$ protein to yield absorbance values of only ~ 0.05 (Rosenberg, R. C., Root, C. A., and

Gray, H. B., *J. Am. chem. Soc.*, **91**, 21; 1975). It is presumably partly for this reason that the nickel ion in urease has remained hidden for so long.

Vanadium is present in high concentrations in the blood cells of certain tunicates, marine animals which feed by filtering seawater. The metal is contained within clusters of vacuoles, called the vanadophores, each about 2 μm in diameter, which are themselves housed in the blood cells or vanadocytes, 8 μm in diameter. The vanadophores, which are pale green, are known to contain vanadium (III) ions and 1.8 N sulphuric acid. But the exact nature of the vanadium ions within the vanadophore is not clear. The difficulty arises because of the fragility of the vacuoles and because of their instability towards oxygen. The vacuoles lyse readily in distilled water to give a dark red-brown haemolysate with a pH of 2.5. Known as Henze solution it is presumed to contain vanadium in the trivalent state. When neutralised the solution turns deep blue, possibly by oxidation to form the vanadyl ion, VO^{2+} . In Henze solution the vanadium is bound to a protein termed homovanadin and to a small organic nitrogenous ligand (Bielig, H. J., *et al.*, *Protides Biol. Fluids*, **14**, 197; 1966). It was hypothesised that in the intact vanadophores the vanadium is likewise bound to a protein and small ligand. Now Carlson (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2217; 1975) has re-examined the problem using proton NMR spectroscopy to probe the vanadium ion within intact vanadophores of the tunicate *Ascidia ceratodes*. Since lysing of the vacuoles offers the opportunity for oxidation, denaturation and artefact formation in the haemolysate it is an advantage to be able to probe the metal ion *in situ*.

The NMR spectrum of whole blood cells exhibits a broad 21-p.p.m. downfield, Gaussian signal which disappears on cell lysis. By comparison of this signal with the sharper signal, also at 21 p.p.m., found in 0.72 molal solution of vanadium (III) chloride in 0.5 M perchloric acid the signal is attributed to vanadophore water protons in rapid exchange with the protons of the water in the co-ordination sphere of vanadium inside the vacuoles. Coupled with the intensity of the signal the broadness argues against the signal being due to protons of a stable vanadium (III) complex. Carlson further points out that as the 21-p.p.m. signal decreases in amplitude and shifts upfield on lysis simple dilution is not occurring but rather oxidation and/or complex formation with a high molecular weight material. He also attempts a quantitative estimate of the vanadium (III) molal concentration by comparing the bulk resonance shifts of water in the spectra

of suitable vanadium (III) standard solutions with the magnitude of the 21.5-p.p.m. signal. This leads to a figure for the amount of vanadium accounted for by the 21-p.p.m. signal and, knowing the total vanadium content from atomic absorption studies, the maximum number of vanadophore vanadium (III) co-ordination sites interacting with ligands other than water *in vivo*. The conclusion is reached that a maximum of one co-ordination site per vanadium (III) ion is left available for binding an organic ligand. This view of a partially complexed vanadium (III) ion within the vanadophore is not in complete agreement with the views advanced earlier by Bielig.

Reviewing the function of the vanadium vacuole in the light of his recent work, Carlson proposes that the vanadocytes have properties not shown by the haemolysate, the system usually studied. It may even be necessary to regard the vacuole as the smallest functional unit. Carlisle (*Proc. R. Soc.*, **B171**, 31; 1968) has shown that intact tunicate blood cells act as oxygen storage and transport units although the blood haemolysate does not oxygenate reversibly. Carlson's work suggests a way out of this apparent contradiction. Further probe studies of the intact vanadocytes will be of great interest. Measurement of the electron spin resonance spectrum and the electronic spectrum using microspectrophotometry are two obvious techniques.

Beyond this, ascidians should have an increasing fascination for chemists in their search for biological functions of metals beyond the first transition series. In his 1968 review, Carlisle reports that many other metal ions besides vanadium are actively accumulated by different species of marine animals, for example, "titanium, chromium, manganese, iron, niobium, and tantalum, certainly, and possibly also molybdenum, zirconium, and tungsten. In all the animals investigated the metals are found in blood cells . . . associated with acidic conditions in which they exhibit a powerful reducing action." It is well known that at low pH the early transition metal ions will condense to form polymeric oxy-anion clusters but only in their higher oxidation states. Goldberg (*Mem. geol. Soc. Am.*, **67** (Treatise, 1), 345; 1957) has shown that these metals are actively accumulated since a lifetime of passive filtration could not pass sufficient water through ascidians to yield the levels which have been reported. The enrichment factors he quotes are spectacular. For example, for marine organisms by dry weight over seawater, titanium is enriched 10,000-fold, vanadium 280,000-fold and niobium 98,000-fold. Very little of the chemistry of these

processes is understood although in future the technological and environmental importance of such scavenging of metal ions will undoubtedly increase.

Hill reaction in the mountains

from Peter D. Moore

WHEN Turesson began his classic work on development of ecologically adapted races within species (*Hereditas*, **3**, 211; 1922), he paved the way for what has proved to be a profitable interaction between genetics, ecology and, more recently, biochemistry. In the early days of such studies, genetic strains could be detected only by structural variation, but recently it has become possible to document more subtle, physiological adaptations to differing environmental circumstances within certain species. Particular attention has been paid to temperature optima of selected biochemical systems from species at different altitudes or latitudes.

A system that has been investigated in several species is the Hill reaction of photosynthesis. This process, which is observed in isolated chloroplasts, when illuminated, involves the movement of electrons from water to an added chemical oxidant (such as potassium ferricyanide) against the chemical potential gradient. The reaction involves the production of oxygen, a process which is thereby demonstrated to be separable from carbon dioxide reduction in photosynthesis.

In 1968, Tieszen and Helgager (*Nature*, **219**, 1066) described experiments with populations of the grass *Deschampsia caespitosa* from arctic (Alaska) and alpine (Colorado) habitats. They measured the response of the Hill reaction (as ferricyanide reduction) to a range of temperatures and observed that the optimum for reaction was approximately 10 °C higher in the alpine population than in the arctic one. This appears to be related to the environmental conditions in the two habitats, for the alpine site would have higher daytime temperatures, whereas survival in the arctic conditions would be favoured by maintained activity even at low temperature. As with the work of Mooney and Billings on *Oxyria digyna* (*Ecol. Monogr.*, **31**, 1; 1961) a degree of flexibility was found if growth conditions were varied, particularly in the alpine population.

May and Villarreal (*Photosynthetica*, **8**, 73; 1974) subsequently examined the Hill reaction in populations of *Taraxacum officinale* from three different alti-

tudes in the Rocky Mountains of Colorado. When assayed at 15 ° and 25 °C populations from high altitudes (3,550 m) had the greatest rate of Hill activity. The reverse was true at 35 °C when the material from the highest site was virtually inactive. The authors suggest that the high rate of the Hill reaction in the elevated sites is an adaptation for fast growth during the short alpine season. This is coupled with a lower temperature optimum at the alpine site. This presupposes that the selection pressure for rapid growth is greater at high altitude than at low altitude. This is conceivable since other pressures, perhaps involving seed production and competition during establishment, may be more significant at lower altitudes. It would be interesting to couple these biochemical investigations with studies of the population biology of these plants in the vein of Gadgil and Solbrig's work on *Taraxacum* (*Am. Nat.*, **106**, 14, 1972). It would be of value to know whether the populations examined had been subjected to predominantly r and/or K selection within their respective habitats, for this could determine the relative survival value of rapid growth.

May and Villarreal collected their material from the field and analysed it within 4 h. They were not able, therefore, to conduct experiments in which growth conditions were varied and in which phenotypic acclimation could be separated from genotypic adaptation. In addition, the apomictic breeding system of *Taraxacum* makes it difficult to know how many separate genotypes are involved in these experiments. It has been demonstrated, however, that even in out-breeding species, such as *Abies balsamea*, the temperature optimum for growth varies considerably along altitudinal gradient (Fryer and Ledig, *Can. J. Bot.*, **50**, 1231; 1972).

It would be unwise to extrapolate from these data to make predictions for other species, however, as the results of Williams, Lazor and Yourgrau (*Photosynthetica*, **9**, 35; 1975) demonstrate. They have taken samples of *Verbascum thapsus* from several latitudes (30°, 47° and 51°) in the United States and also at several altitudes within the same latitude (in Colorado, of course). In contrast to the results of previous workers, they found that the high altitude populations had lower rates of Hill activity and there was no clear cut difference in temperature optima. In their latitudinal studies they found that low latitude populations showed higher activity than those of high latitudes. Thus the negative relationship between Hill activity and length of growing season shown by May and Villarreal for *Taraxacum*, does not hold for *Verbascum*. The very broad range of temperature over which *Verbascum* is

capable of maintaining high rates of Hill reaction is a notable feature of these studies. This could contribute to the success of this species as a weed of disturbed habitats in North America as well as in Europe and many other parts of the world.

Two main points emerge from these pieces of work. First, differences in Hill reaction rate and differences in the temperature optima for Hill activity exist between isolated plant populations, and there is evidence suggesting that these differences are genetically based. Second, it has become apparent that further advances in the understanding of these processes can only be achieved by making use of controlled environment growth experimentation. This is needed to differentiate between genetic and phenotypic adaptation within test populations and to document changes which may occur in the rate of Hill reaction with season or with advancing physiological age of the tissue being tested. □

Large scale shell model calculations

from N. MacDonald

A meeting on Large Scale Shell Model Calculations was held in Glasgow on August 27-29.

THE shell model plays a major role in the understanding of nuclear structure. Most of the particles in the nucleus are treated as inert, while the remaining few active particles are, to a lowest approximation, treated as moving independently in a common central potential. This approximation does not get one very far, and one calls on an effective interaction between pairs of active particles.

Two essential questions are thus raised. One is how to devise an effective interaction that can be related to the known features of interactions between free nucleons. The other is how many of the particles are to be treated as active, and how many states are to be regarded as accessible to them. About three years ago a substantial advance was made in handling moderately large numbers of particles. The technical problem involved is that of diagonalising very large matrices. R. Whitehead recognised that the key to this lay in the revival of the Lanczos algorithm, an iterative method involving repeated multiplication of an arbitrary vector by the matrix. When the vector has the form of a shell model wavefunction and the matrix

is that representing the two-body effective interaction, this iteration can be performed without ever storing the elements of the matrix.

The Lanczos method is the essential feature of the Glasgow shell model code, which has been used for very complete surveys of the nuclei from mass 17 to 39, over which the active particles fill the s-d shell. The meeting was held to review the progress made in these surveys, and the status of the shell model generally.

B. Barrett (University of Arizona, Tucson) reviewed the theory of the effective interaction. The problem is to go from a known interaction and a large set of states to an interaction yielding a good approximation when restricted to a much smaller set. The method used is perturbation expansion. Convergence is controlled by the extent to which states of the excluded part of the large set overlap in energy with those of the small set. For the s-d nuclei these intruder states undoubtedly lie at embarrassingly low energy. More sophisticated ways (double partition) of dividing up the large set of states may yet yield an effective interaction with a sound foundation, but at present shell model calculations are best tackled in a less ambitious spirit.

This means starting from a subset of the available data on a range of nuclei and determining the interaction that fits this best. Then one can test it on the remaining data. This process can start from a simple form of interaction, as in work on the s-d shell reported by P. W. M. Glaudemans (University of Utrecht) and other Dutch workers, who start from a surface delta interaction. They vary seven parameters to provide a preliminary estimate, and then carry out an iterative variation process on all 63 matrix elements. One may instead elect to vary all the two-body matrix elements without any constraints. The most ambitious attempt of this kind was reported by B. H. Wildenthal (Michigan State University). He used the Glasgow code in an unsuccessful attempt to locate such a best fitting interaction to data from the whole shell. Failing this, he was able to provide separate interactions for the upper and lower halves of the shell. These supersede the earlier Michigan interactions found by fitting data over smaller sets of nuclei with the Oak Ridge codes.

The next step into larger spaces, as for example nuclei of mass 40 to 70, goes beyond our present powers of computation. It becomes essential to truncate the space used. It was hoped that with the Glasgow and Michigan results for the s-d shell one might be able to devise a trustworthy truncation scheme. One approach reported by

P. Manakos (University of Darmstadt) and J. Millener (Oxford University) uses the SU3 scheme, which relies on the approximate validity of certain symmetries in nuclear states. Another (J. Morrison, University of Glasgow) is to employ a deformed rather than a spherical common potential. A danger which emerged in studies of truncation, in particular in those reported by A. Watt (University of Glasgow), is that the best strategy may depend very much on the interaction used. But in any higher shell the interaction may be quite different from those used in the s-d shell. A consolation is that in studying this question one learns quite a lot about the nature of the states ground out by the mammoth computations.

The most mysterious feature of the Lanczos method in its nuclear applications is the extremely rapid convergence of the iterations. Almost independently of the size of matrix, 50 to 100 steps suffice to get the desired low states. R. Whitehead (University of Glasgow) presented various results related to this. One is the formal relationship, by way of the technique of Pade approximants, between the Lanczos method and the inverse iteration method. Another is that performing 50 multiplications by the energy matrix, starting with an arbitrary vector, is related to evaluating the first 50 moments of the energy in an arbitrary state. Techniques for studying low moments, which were discussed by O. Bohigas (Orsay), could conceivably be extended to approach the shell model in a different way. □

European earth scientists seek closer collaboration

from A. J. Smith

The meeting of European Geological Societies (MEGS) organised jointly by the Geological Society of London and the University of Reading was held at Reading from September 8 to 12.

THE theme of the meeting, which was attended by about 400 earth scientists, was 'Europe from Crust to Core', and early announcements expressed the hope that geologists and geophysicists "would discuss the crucial scientific and socio-economic questions affecting European geology in the next 100 years". The programme promised that the theme and ideal might be achieved and in the event, thanks to excellent organisation and a warming by those

present to the concept of a collective European image, the promoters can feel well satisfied. In their opening remarks Sir Kingsley Dunham (Institute of Geological Sciences, London) and P. Allen (University of Reading) urged participants to cross political and disciplinary boundaries for the benefit of geological science, Europe and the rest of the world, a theme to which J. M. Harrison (UNESCO, Paris) returned on the closing day of the meeting.

The programme was built upon Stille's concept of Europe's crustal framework, with thirteen keynote addresses, numerous shorter contributions and ample time for formal and informal discussion. The opening keynote address by V. E. Khain (Moscow State University) was concerned with the new tectonic map of Europe which is to appear in 1977. He reviewed the wealth of geological information which has become available since the production of the first tectonic map of Europe less than a decade ago. Both he and Janet Watson (Imperial College, London), in her address on the evolution of the European craton, drew attention to the strong influence of a Precambrian framework on the succeeding development of the European crust. Major structural lines, once started, have reasserted themselves and influenced succeeding events. Other keynote speakers and contributors made clear that ocean floor spreading did not offer a panacea for Europe's lithospheric history and, instead, that gravity tectonics, often along old lines, frequently offer a more satisfactory explanation. A. Kvale (University of Bergen) pleaded for more work to increase the scientific value of plate tectonic models applied to the European Caledonides, while W. Krebs (Braunschweig Technical University) questioned the existence of any Palaeozoic 'oceanic' crust anywhere in central Europe, preferring the concept of repeated metamorphism and plutonism caused by ascending mantle diapirs. H. Ramberg (Uppsala University), Krebs and many others saw the driving force of folding, thrusting, allochthonous gliding and nappe transport as resulting from the potential relief energy between rising zones and synchronously subsiding adjacent troughs. Sir Peter Kent (Natural Environment Research Council), dealing with new knowledge from the North Sea, drew attention to the relationship between freer movement of plates in North-western Europe from mid-Cretaceous times consequent on the parting of the crust in the Atlantic replacing the stretching of marginal continental crust. He also drew attention to the need for a reappraisal of accepted theories for the origin of the Permian evaporites. A. Aubouin (Université

Pierre et Marie Curie, Paris), in his summary of the Alpine orogeny of the eastern Mediterranean, while accepting the 'classical' plate tectonic approach, though without the need for entrapped microplates in the eastern Mediterranean, stressed the need for more work to explain the numerous enigmas. E. Niggli (University of Berne) instanced the problem of the depth of crystallisation of blue schists and the possibility of tectonic stripping of cover.

Geophysical topics concerned with the deepest crust, mantle and core naturally ranged beyond the limits of the European continent. Several geophysicists drew attention to the contrasting crustal thickness between adjacent European areas—the non-sedimentary crusts beneath Cainozoic depressions being some 30 to 45% less than beneath the Hercynian massifs.

Many speakers stressed the role of the geologist as a forecaster as well as a recorder of events. G. F. Mitchell (Trinity College, Dublin) made much of this in his Quaternary review, as did M. Arnould (Ecole National Supérieure, Paris) and G. Luttig (Geological Survey, Hanover, FDR). Geothermal energy, the disposal of radioactive wastes, environmental and conservation topics were discussed in the European context. Speakers both in the main conference and at the meet-



A hundred years ago

IT is rather disappointing that Capt. Young's Arctic Expedition in the *Pandora*, which arrived at Portsmouth on Saturday, should have returned home prematurely without accomplishing any part of the work for which it was organised—the discovery of additional Franklin relics and the complete navigation of the North-west Passage. Under the circumstances, however, Capt. Young has adopted the wisest possible course. Better that the expedition should spend a comfortable winter at home, and set out early next year to renew the attempt in which they have just been baffled. Disco was reached on August 7, Upernivik on the 13th, and Cape York on the 16th, after a splendid passage through the much dreaded Melville Bay. Carey Islands were visited to deposit letters for the *Alert* and *Discovery* and to obtain a despatch from Capt. Nares, as previously agreed on. The despatch, however, was not discovered till the return voyage.

From *Nature*, 12, 539, October 21, 1875.

ing of the Commission on the Tectonics of Ore Deposits, which met concurrently with MEGS, described the value of ERTS (Earth resources technology satellite) imagery in a greater understanding of Europe's structure and in the search for mineral resources. Pleas that geologists generally should pay more attention to the special needs of engineers and planners were common, and the need for more mathematical, chemical and experimental approaches to earth science was examined.

European geologists were reminded of their obligation to the rest of the world in more than just an economic sense: G. Jenkins (University of Canterbury, Christchurch, New Zealand) reminded them that stratotypes, the key to the worldwide geological division of time, were often first described in Europe, and that to aid worldwide

correlation more must be researched in Europe and published.

A perhaps oversimplified view of the meeting might be that extremely useful summaries of the state of European geology were given, showing that Europe still presents fundamental questions in need of answers. Seeking such answers can occupy European geologists, geochemists and geophysicists for a long time to come. Time, particularly in respect to resources, will not wait for geologists any more than for the rest of mankind, and the participants recognised that closer collaboration between European earth scientists is urgently needed.

About a year ago correspondence in *Nature* referred to MEGS under the heading 'European earth scientists disunite', suggesting that a schism had opened between European 'geologists' and geophysicists. In this context it is

a pity that so few geophysicists attended MEGS for they would have enhanced the meeting. The Reading meeting showed that European earth scientists sincerely desire to act in concert for the benefit of Europe and the world. The UK initiative which created MEGS was warmly appreciated. The establishment of a European Geological Society was discussed and received wide support but no final decision was reached. Instead it was decided to set up an interim committee composed of representatives of a number of the participating countries to promote a MEGS 2 (representatives of both the Netherlands and France indicated a desire to host such a meeting), to cooperate with existing international and European bodies and to encourage closer communication between European earth science societies. □

CONTRARY to widespread belief, although the steady state theory of the Universe may be dead it refuses to lie down, and a small band of enthusiasts continues to try to breathe life into the presumed corpse. But the chances of a miraculous resuscitation do not look too good, as the appearance of two papers together in the same issue of *Mon. Not. R. astr. Soc.* shows. In one of these contributions (172, 623; 1975) P. K. Das of the Tata Institute develops further the model of QSOs as compact, highly gravitationally redshifted objects that he and J. V. Narlikar put forward recently (*Mon. Not. R. astr. Soc.* 171, 87; 1975); although Das does not mention the steady state theory, it seems a reasonable assumption that any colleague of Narlikar's who discusses possible non-cosmological contributions to the redshifts of QSOs has the concept not too far towards the back of his mind.

This model has a great deal of interest for theorists and students of relativity, whatever its application in the real Universe, and consists of a static, spherically symmetric distribution of a mass M in a radius R (Das uses dimensional units), in hydrostatic equilibrium, obeying Einstein's equations and divided into two regions, core and envelope. The earlier paper from Das and Narlikar developed equations for the isothermal core-adiabatic stable envelope configuration and discussed some implications for redshifts; in his new work Das concentrates on a discussion of how light propagates from both the surface and interior of such an object, and the effect of this on the angular diameter seen by an external observer.

The observed features of such an object depend on whether R is greater

One step forward and two back for the steady state

from John Gribbin

than or less than $3M$, and the ratio R/M is reduced by 'stiffening' either the core or envelope, that is, making the model more relativistic. For light emitted from the surface, as the core or envelope equation of state is stiffened the angular diameter decreases from its Euclidean value; for sufficiently stiff core-envelope combinations, the back of the source becomes visible, with the entire surface eventually being mapped into a thin ring.

But the source need not appear as a ring, and light emitted from the interior can play a part in determining the overall appearance. Combining the effects on light from the core and from the surface with the earlier investigation of redshifts, Das is able to produce an angular diameter-redshift ($\theta(z)$) relationship. He finds that as the core-envelope combination is stiffened a plot of θ against z shows a minimum, which shifts to higher z as the stiffening increases, and "it is interesting to note that θ calculated on the basis of cosmological redshift hypotheses also shows a minimum at comparable values of z ".

The properties of this model do depend critically on the equation of state, and for pressure as high as half the energy density the angular dia-

meter is constant irrespective of surface area, with multiple imaging taking place. Of course, radiation from the interior will be absorbed by intermediate material *en route* to the observer, and investigation of the expected absorption feature is the logical next step in developing this intriguing model. Intriguing though it is, however, it is a little artificial, and its relevance to the real world must be in even greater doubt in view of the paper by V. K. Kapahi (ironically, also of the Tata Institute) in the same issue of *Mon. Not. R. astr. Soc.* (172, 513; 1975). He has looked at angular size counts of extragalactic radio sources, using the 3CR catalogue, and finds evidence of evolution in source properties even when QSOs are excluded from the sample. This is already difficult to reconcile with steady state ideas, and the same sort of evolution also seems to be present when QSOs are analysed separately, which suggests rather strongly that QSOs really are part of the same family as other extragalactic radio sources.

The best explanation of these variations is to fit them to a simple Einstein-de Sitter cosmology in which the local radio luminosity function (RLF) steepens considerably at high luminosities, the comoving density of high luminosity sources increases with z in a manner similar to that implied by number-luminosity ($\log N - \log S$) studies and the V/V_m test for QSOs, and mean physical sizes of radio sources evolve with z approximately as $(1+z)^{-1}$. This important confirmation of the pattern suggested by the earlier studies hints strongly that even if a way can be found of mimicking QSO properties in other ways, the cosmological interpretation stands better than ever before. □

Computer supplement

How Babbage's dream came true

M. V. Wilkes

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Charles Babbage dreamed up a form of computer in the 1830s. His ideas lay dormant until digital machines became a reality in the 1940s. Professor Wilkes, one of those responsible for the implementation of the digital computer, describes some of the prehistory and early history.

COMPUTERS have a history and a prehistory. If we confine ourselves to electronic computers the first historical date in the computer era is February 18, 1946 when the ENIAC was formally inaugurated at the Moore School of Electrical Engineering in the University of Pennsylvania. Nine months earlier, in May 1945, the Automatic Sequence Controlled Calculator had been set going at Harvard University. It was not an electronic machine but was based on the use of rotating shafts, electromagnetic clutches, relays, and so on. It had, however, the distinction of being the first fully automatic digital computer to be completed. Its origins can be traced back to a report written by Howard H. Aiken in 1937. The machine was built by International Business Machines (IBM) with Aiken's vigorous co-operation. A number of machines using telephone relays for computing purposes were built. As early as 1938 there had been interest in this subject at the Bell Telephone Laboratories and a number of special purpose devices had been built and used. This work, however, did not culminate in the construction of a completely automatic calculating machine until 1946. Zuse also worked on relay computing devices in Germany during the latter part of the war. Randell¹ provides documentation of this period.

The prehistory of the subject formed the life's work of just one man, Charles Babbage (1792–1871). Although he never completed his machine Babbage had all the ideas and L. J. Comrie when writing for *Nature* a review of a book describing the Automatic Sequence Controlled Calculator had the happy thought to entitle it "Babbage's Dream Comes True". I have adapted this title for the present article.

Babbage began to think about calculating machines in about 1812 or 1813 at a time when he was still a student at Cambridge. His interest had been caught by a very ambitious project for computing mathematical tables started in France during the time of the first Republic. The principle adopted in this project was to compute accurate values at rather widely spaced values of the argument, and to produce values at the required tabular interval by systematic interpolation or subtabulation. Babbage saw that this could be done by a machine which he called the Difference Engine. Not only would this have calculated tabular values, but it would also have prepared the moulds from which stereo plates for printing could be cast. This was an important point and Babbage thoroughly appreciated the importance of avoiding copying and typesetting errors.

Babbage became very enthusiastic about the Difference Engine and successfully communicated his enthusiasm to the

British Government, who gave him financial support. All went well to begin with and he spent a number of years happily engaged in supervising the construction of the engine. Unfortunately, however, a high rate of progress was not maintained and difficulties arose between Babbage and his engineer. Eventually the project had to be abandoned without there being anything to show for it.

Babbage's mind was, however, working on generalisations of the Difference Engine and suddenly around 1834, when he was 42 years old, he began to perceive how a truly general purpose calculating machine could be made. One idea followed another in rapid succession. On September 18, 1834, he wrote to his close friend John Herschel, the astronomer, who was then working at the Cape of Good Hope: "I have begun to criticise all I have done and to consider it merely as the bricks of a better fabric. In the first place I have simplified and can, therefore, again generalise. . . . In short I see my way on towards turning the whole of analysis into wheel work as far, at least, as numerical results go, and this will at last come"². The intellectual excitement that Babbage was experiencing at that time is well brought out in an earlier letter to Herschel: "I suppose like myself you have never one hour out of the twenty-four to spare; yet I should like, if possible, to catch one to discuss with you some of the *stray* questions about which I cannot resist indulging myself as a refreshment from the Analytical Engine"³. He goes on to mention several topics, such as the theory and practice of cutting tools, how to make a real automaton, and so on.

The machine that Babbage named the Analytical Engine was—or would have been had it been built—a true, automatic digital computer. Like a modern computer it was to have a store in which numbers could be held, and a processor known as the "mill" in which the arithmetic operations would be performed. For the control of the calculations, Babbage at first proposed to use a drum with moveable studs, but almost at once he took the important step of deciding to adopt the Jacquard mechanism that had then come into general use for controlling looms. His ideas developed very rapidly and by the end of 1837 he had drafted an account setting them out in some detail. For the rest of his life Babbage worked on the design of the Analytical Engine, improving and refining his ideas, but his account of 1837 contains all the essentials (see ref. 1).

For some reason or other Babbage did not publish this account; in fact there is very little in his published writings about the detailed way in which his ideas would be implemented. He described the functions of the store and the mill, but he did not even introduce a term to describe what we would now call the control unit. A good deal of information was, however, published about the general principles on which the Analytical Engine worked and the type of calculation for which it could be used. In 1843 Babbage gave a series of presentations in Turin. An account of these was written by Menabrea and published in a Swiss journal. The paper was translated from

the original French by Lady Lovelace who added extensive notes, written under Babbage's close supervision. The idea of repetitive loops within a calculation was explained, but the actual way in which they were set up was not described. Lady Lovelace gives a number of what appear to be programs, but close examination shows that only the arithmetic operations are described; the repetitions are indicated by comments or by statements in the text that certain series of operations are to be repeated so many times. Babbage proposed to use separate Jacquard mechanisms to control operations and variables; these would step independently and go through independent cycles. For some reason he never arrived at the idea, so familiar to us, of an instruction consisting of an operation code associated permanently with one or more addresses. It is, however, clear from his notebooks and other unpublished writings that he gave very detailed thought to the sequencing mechanism⁴. Associated with each Jacquard mechanism he proposed to have a "card counting apparatus" or "repeating apparatus". This

Charles Babbage (1792–1871)



Let it be granted that in his life there was much to cause disappointment, and that the results of his labours, however great, are below his powers. Can we withhold our tribute of admiration to one who throughout his long life inflexibly devoted his exertions to the most lofty subjects? Some will cultivate science as an amusement, others as a source of pecuniary profit, or the means of gaining popularity. Mr. Babbage was one of those whose genius urged them against everything conducive to their immediate interests. He nobly upheld the character of a discoverer and inventor, despising any less reward than to carry out the highest conception which his mind brought forth. His very failures arose from no want of industry or ability, but from excess of resolution that his aims should be at the very highest. In these money-making days can we forget that he expended almost a fortune on his task? If, as people think, wealth and luxury are corrupting society, should they omit to honour one of whom it may be truly said, in the words of Merlin, that the single wish of his heart was "to give them greater minds"?

From W. S. Jevons's obituary notice of Charles Babbage (*Nature*, 5, 28–29; November 9, 1871).

would be loaded with an integer which would be decremented whenever a card was read. When the counter liquidated ('running up' was Babbage's term) an appropriate action would ensue. Babbage is not anywhere fully explicit about how the system would work and I doubt if he ever entirely made up his mind between the various alternatives. He proposed to systematise the internal control by making use of "barrels", or drums with projecting studs, a system that offers a striking parallel to microprogramming.

Reading Babbage makes one realise what an important advance was the development of the modern concept of a program as something which can exist as a valid statement of an algorithm independently of the computer on which it will run and about which things may even be proved. Part of the breakthrough of the stored program computer was the development of a language—initially machine language only—in which programs could be written. If Babbage had described in formal terms the role played by his card counting apparatus and had introduced a notation for the way in which the Jacquard cards were punched, he might have taken this vital step; however, the fact that he had independently stepping Jacquard mechanisms to control operations and variables would have made the programs difficult to follow, and they would not have borne much resemblance to modern programs.

One must ask why, in the end, Babbage had so little to show by way of practical achievement. I think that he might well have hoped to finish the Difference Engine. The ideas were sound enough, as was shown by the fact that another implementation of them, by George and Edward Sheutz, was successful; however, that was a good deal later, in 1855, and the techniques of mechanical engineering were developing rapidly at that period. Much of the responsibility for the failure of the Difference Engine must fall on Joseph Clement, whom Babbage employed to be in charge of the construction. In April 1828 Herschel, who was looking after things during Babbage's temporary absence in Italy, clearly thought that the progress being made was inadequate in view of the amount of money being spent. At that time Clement was employing 10 people on the project⁵. Babbage, however, continued to have full confidence in Clement and it was not until five years later that they finally fell out.

The Analytical Engine was another matter. I doubt if it would have been possible for Babbage to have produced a full scale machine that would work at all reliably. To do so using the mechanical principles he had in mind would be far from easy today. In view of what had happened to the Difference Engine, there was little prospect of his getting the necessary financial support to set up a proper project. Babbage was, however, prepared to spend his own money and always had people in his employ; after his death his younger son carried on the work for some time. As a result many drawings were made and some pieces of the Analytical Engine were constructed; these are now to be seen in the Science Museum at South Kensington.

Babbage's real achievements were intellectual, and they remained buried in his notebooks. In March 1839 he did indeed draft a plan of a work entitled *The Science of Number Reduced to Mechanism*⁶. He revived this project at various times and it is hard to see why he did not persevere with it, particularly since he was an accomplished writer and published extensively on other subjects. If he had done so he might well have shown the way for someone else to put his ideas into practice. As it was they were lost and what was of value in them had to be re-discovered.

We must, I think, conclude that, although a mathematician and a man of science, Babbage was, as far as calculating machines are concerned, an inventor at heart. That is to say he saw achievement in terms of the successful construction of a device and not in terms of the publication of ideas. Inventors are vulnerable to the hazards of the world and as likely as not end by becoming bitter and disappointed men. Babbage had more than a twinge of such feelings, but fortunately the Analytical Engine, although it occupied a large part of his life, was not his sole preoccupation. His other interests were wide and varied

and he led an active social life. He could be tiresome and self-important, but he had many friends and was by no means lacking in humanity, as anyone reading his autobiography will appreciate.

When I became interested in computing in the years immediately before the Second World War, L. J. Comrie and others were showing great ingenuity in using commercial accounting machines for scientific purposes and they took great delight in exploiting their special features in new ways. The climate of opinion was, however, distinctly hostile to the production of special digital machines for scientific calculation, either by the adaptation of commercially available machines or otherwise. On the other hand, various analogue devices were coming along; these included the differential analyser, announced by Bush in 1931, and various linear equation solvers. The scientific world was prepared to give these a sympathetic reception and it suffered the inevitable disappointment. In fact, when the EDSAC began to work in Cambridge, one of my tasks was to persuade people that it was not like another analogue device with its own esoteric techniques, but that it handled proper numbers in the proper way. Fortunately, I was helped by the fact that the second program run on the EDSAC was one for the listing of prime numbers, something manifestly respectable from an arithmetic point of view.

The EDSAC began to work in May 1949 and other early computers came into operation at about the same time. These were the first stored program computers—the ENIAC had been programmed by means of a system of plugs and sockets and switches—and the development of the new subject of programming then became possible. As might be expected, different groups followed their individual ways and there was a good deal of diversity. That was not only because the computers had their own instruction sets but also arose for more or less accidental reasons connected with the background and attitudes of the staffs involved. Computer peripherals were in a primitive state of development and the capabilities of a computer were much coloured by what it happened to possess in this respect. This was particularly true because all the early computers had very small high speed memories. The Pilot ACE at the National Physical Laboratory (NPL), for example, used punched cards for input and output, and it was possible to punch 12 binary numbers on a card, one on each row. Punched cards could thus be used as an auxiliary memory and the machine was very suitable for performing matrix calculations. The NPL, therefore, took a lead in that aspect of computing, with consequences that remain to this day. In Cambridge we had punched paper tape for input and output, and a mechanical read-only memory from which a set of initial orders could be copied into the high speed memory to control the reading of the program tape. We were, therefore, led to develop a format for instructions that may be regarded as the forerunner of later assembly languages. At Manchester, they had a magnetic drum as well as a high speed memory from the very beginning, and they therefore developed methods of programming that exploited a large auxiliary memory earlier than the rest of us. This diversity was very helpful to the development of the subject, and we all learnt much from comparing notes. Those who were active in the small computer world of the late 1940s and the 1950s will remember the regular colloquia held in Cambridge on certain Thursday afternoons, which provided a good opportunity for the experts to compare information. They also served the purpose of those who wished to inform themselves about what was going on at a time when published material was scarce and contact with one or more of the established centres was essential.

It came as a surprise to me personally to find that it was so difficult to get programs right. In Cambridge there was much emphasis both on programming aids to debugging and on methods of constructing programs that would be relatively fool-proof. The use of previously tested subroutines was one such method, and we recommended the construction of a program by means of nested, closed subroutines with program para-

meters; if one substitutes the words 'procedure call' for 'closed subroutine', we have here one of the central tenets of structured programming. A consequence of the adoption of these methods was that we found little need to draw flow diagrams, and I have always felt that the motto on the structured programming banner should be "flow diagrams considered harmful".

Along with the development of programming techniques went the development of computer applications. An early problem to be solved on the EDSAC was one proposed by R. A. Fisher⁷. He wrote to me early in 1949, before the EDSAC was working, saying in characteristically barbed fashion that he understood that our fine new machine was not going to be used only for wave mechanics and that he would like to have a numerical solution of a certain second order nonlinear differential equation with two point boundary conditions. I do not think that he had much hope that we would produce the solution, but one was, in fact, obtained by D. J. Wheeler as part of his thesis work. Fisher was duly impressed and he eventually published the solution with a suitable acknowledgement.

Atomic calculations were, of course, an obvious field of application for an electronic computer, although we realised that the early machines would be somewhat lacking in capacity. I remember one day accidentally running into S. F. Boys at Imperial College. We had known each other in Cambridge before the war and he asked me what I was doing now. I told him about the EDSAC under construction and rather to my surprise he began to explain to me why such a machine would not be of any use for the type of atomic calculation that he was interested in. I kept my counsel, but later, when Boys had returned to Cambridge and became an enthusiastic user of the EDSAC, he was only too ready to confess how wrong he had been. The point that he had failed to grasp was that a digital computer could perform logical as well as arithmetic operations; the EDSAC had one order for performing a logical operation, namely, that of bit by bit Boolean multiplication of two binary numbers. This was known as the 'collate' order and Boys found that it made all the difference.

I was more than willing to tell people about the EDSAC, but I never made any attempt to sell them the idea of using it if I met with sales resistance. To do so was unnecessary since there was no shortage of users. At first, professional programmers did not exist and people had either to learn to do their own programming or persuade someone who was working with a machine to do it for them. Our own research students were the best ambassadors. Computer applications in X-ray crystallography were pioneered in Cambridge by J. C. Kendrew, then a junior member of the staff of the Cavendish Laboratory, and J. M. Bennett, a research student with us. This led in due course to the calculations for the structure of myoglobin that were done on EDSAC 2. It was through contacts of this kind that the use of a digital computer spread in Cambridge, and I am quite sure that the same is true of other places.

In the very early days it was possible for the management of a computer centre to follow in some detail what all the users were doing. We had in the laboratory a body known as the Priorities Committee before which all applicants for computer time would appear to explain their problem. In spite of its name, the Priorities Committee was essentially a technical committee, and the members would discuss with the applicant the best way to go about his work, the best numerical procedures to use, and so on. From time to time, the applicant would come back to tell the committee how he was getting on. I found these regular meetings of great interest, since they brought me into touch with a wide and ever expanding variety of subjects, as more and more research workers realised that a computer could be of assistance to them. In the course of time, much of the work of the committee became routine, but it still continued to discuss the more interesting projects.

I have mentioned that one of the very early programs to be run on the EDSAC was one for listing prime numbers. This particular program worked by the crude method of dividing each odd number in turn by all the odd numbers not greater

than the square root. The division was done by repeated subtraction since the EDSAC had no divider. It took about 10 minutes to compute and print the first 170 primes. Wheeler later wrote a program that was essentially a mechanisation of the sieve of Eratosthenes. The EDSAC had a cathode ray tube monitor on which the contents of the high speed memory could be displayed in raster form. It was possible to show 16 35-bit words at a time, that is, 560 bits altogether. In Wheeler's program the odd numbers were represented in order by binary digits. To begin with, all these digits were ones, indicating that all the numbers were present. As the sieve operated and numbers were eliminated the ones were replaced by zeroes. The speed of the machine was such that it was possible to watch this happening on the screen.

J. C. P. Miller, who joined the Laboratory at the beginning of 1950, had a long-standing interest in prime numbers and he and Wheeler set about seeing whether they could break the record for the largest known prime number. This was the Mersenne number, $P = 2^{127} - 1$, which had, some 75 years before, been shown by Edouard Lucas to be prime. Miller and Wheeler prepared a routine for testing the primality of numbers of the form $kP + 1$ and soon found ten values of k that gave prime numbers. On June 7, 1951 they wrote a letter to *Nature* reporting this fact⁸ and giving the largest known prime number as $934P + 1$. They added a note in proof (October 8) giving two even larger primes, the larger of which, $180P^2 + 1$, was the largest then known. They added that A. Ferrier, using a desk machine, had just demonstrated the primality of another large number, and that this was the second largest known prime. Though we were glad that the EDSAC had broken the record, we had much sympathy with Ferrier, who after great labour with a desk machine, had had the trophy snatched from his hands at the last moment by the march of technology. In another way we were fortunate. The prize, by all justice, should have gone to Manchester University, where somewhat earlier a baby model computer had been demonstrated. This was very limited in that it had no reader for punched cards or paper tape,

and no printer, and it could only perform the operation of subtraction; moreover, its memory was minute. Nevertheless, with great skill, a program for testing Mersenne numbers for primality was written, and quite a lot of numbers were tested. Alas, none of them were primes.

Ryle has related in his Nobel address⁹ how the development of radiotelescopes working on the principle of aperture synthesis depended on the availability of adequate computing power. I had a particular interest in anything to do with radio, since my own graduate work had been in the field of radio wave propagation and had been done at the Cavendish Laboratory. Pilot experiments in aperture synthesis were done with EDSAC 1 and EDSAC 2 and showed that a more powerful machine would be needed to handle routine reductions for a large telescope. Fortunately, such a machine was planned to come into operation in 1963 and I was glad to assure Ryle that the time would be available. When the telescope came into operation, there was a standing instruction in the laboratory that the routine reductions for it should have priority over all other work. In the ordinary way, this was not important, since the amount of time required was not great, but it did mean that, when the service was disrupted by maintenance or for other reasons, the telescope came first. It was one of Ryle's colleagues working on optical astronomy who made the remark in a lecture that the telescope was no longer the most important instrument used in astronomy, having been displaced by the computer. That was a clear exaggeration, but it showed what had been achieved in the space of a few years.

¹ *The Origins of Digital Computers: Selected Papers* (edit. by Randell, B.), (Springer, Berlin, 1973).

² Royal Society, London, HS 2 287

³ Royal Society, London, HS 2 305

⁴ Wilkes, M. V., *Babbage Memorial Meeting* (British Computer Society, London, 1971)

⁵ Royal Society, London, HS 2 225.

⁶ *Buxton Papers*, Museum of the History of Science, Oxford.

⁷ Fisher, R. A., *Biometrics*, 6, 353 (1951)

⁸ Miller, J. C. P. and Wheeler, D. J., *Nature*, 158, 838 (1951).

⁹ Ryle, M., *Les Prix Nobel en 1974* (Stockholm, in the press)

Whatever happened to the Stantec Zebra?

David Davies

University central computing needs in Britain are provided for by an increasingly unified set of machines. The next step is likely to involve more comprehensive networking.

BRITISH universities, with about 250,000 students, 40,000 of these at the graduate level, have roughly £58 million of capital equipment on their premises in the form of central computers, and the annual expenditure on hardware, buildings and recurrent costs runs at about £12 million.

Central policy on large computers is the concern of the Computer Board for Universities and Research Councils, which recommends action to the Department of Education and Science on university computing facilities; the board, under the chairmanship of Dr A. H. Chilver (Cranfield Institute of Technology) comprises eight members. It further gives advice to the research councils, although it has no control over their choice. It also reviews longer term policy on computers, which means these days that it does a fair bit of persuading universities that they will have to live with what they have

got for a year or two longer than expected. In the most recent report from the board (in August) broad hints were being dropped that university computers should have a lifetime of 10 years or more, "longer . . . than has been usual in the past". The problem the board faces is that much of its money is committed years in advance and any governmental squeeze is therefore difficult to apply in an even-handed way.

Time was when the university computer laboratory was a place where universities learnt how to build computers and research workers learnt how to operate them. Less than 15 years ago I can recall working in a computer laboratory where the staff used to leave a pile of chasses on the bench each night when they went home. If the machine went wrong, you ran a special program which produced a high pitched note through a loudspeaker. You took a mallet, banged the chasses one after the other, and when the pitch changed you knew you'd found the dud one, which you then replaced. The staff topped up the valves on the dud chassis in the morning.

All that has, alas, gone and in retrospect the turning point at which quirky originality had to be sacrificed to reliable uniformity was the publication in 1966 of the Report of the

Table 1 Recommendations of the Flowers report

University	Had in 1965	Flowers recommended	Has now
Cardiff	Stantec Zebra	Elliott 503	ICL 4/70
Sheffield	Mercury	ICT 1907	1907 and 1906S
Belfast	Deuce (borrowed from US Navy)	ICT 1905	ICL 1906S
Keele	(Private property of Professor McWeeny)	ICT 1903	ICL 4130
Leicester	Elliott 803	Add an Elliott 503	Cyber 72
Aberystwyth	IBM 1620	Elliott 4100	Two ICL 4130s

Flowers Working Group on Computers for Research. The group, charged with assessing university and research council needs for five years, found a wide diversity of incompatible machinery, most of it inadequate either in size or reliability for the burgeoning demands for computer time. The report correctly noted that many scientists were desperate for computer time—"Swansea will shortly be accessible from Aberystwyth only by private car, and that requires 2½ hours driving over mountain roads", "one man [at a London college] found it quicker to compute in Canada, flying there and back at frequent intervals", and so on. Remote terminals were only just coming in; Project MAC with an IBM 7094 at MIT was described admiringly.

Flowers urged that three very large computers be installed in London, Manchester and Edinburgh, which would serve as regional centres. Individual universities should have better computing facilities and compatibility with the regional computers. "The system would thus form an integrated whole." Some of the recommendations of the report are shown in Table 1. Expenditure was expected to be £20.5 million for universities over a period of five years.

The "very large machines" for the regional centres would have to be American. There was an interesting discussion in the report of the problems of keeping up with IBM, but it was clear that the IBM 360/92 and the CDC 6800 were the only real competitors.

The government accepted almost entirely the generous and detailed suggestions of the Flowers report; because of the "economic situation" it would take six years instead of five to implement the recommendations. The three regional centres were set up and a fourth, well equipped centre aimed at providing a focus for the universities in the south-west (Bath, Bristol, Cardiff, Exeter and the University of Wales Institute of Science and Technology—UWIST) is in process of being established at Bath (see Table 2). In addition other universities have been virtually re-equipped since 1966. A summary of computing equipment installed or ordered for all universities is given in Table 3. The Computer Board says, a touch laconically, "without any undue complacency, we feel the aims set out in Flowers Report have, to a large extent, been achieved".

What Flowers could not foresee, and what has added to the importance of the regional centres, has been the emergence of remote terminals as a way of life. Most universities are either on a network such as the South-West network, or possess Computer Technology Limited 'Satellite One's which for £20,000 put the universities in touch, by phone line, with a

Table 2 Equipment at Regional Centres

Centre	Equipment
Edinburgh	2 × ICL 4/75s; IBM 370/158; ICL 2980 (on order)
London	CDC 7600/6600/6400; Cyber 72
Manchester	ICL 1906A; CDC 7600; Cyber 72
Bath (not officially a Regional Centre)	2 × ICL 4/50s; ICL 2980 (on order)

regional centre. No more 2½ hour drives through the Welsh mountains; even the tiny and remote St David's, Lampeter now calls up Swansea on its Satellite One.

The health of British university computing is very much intermingled with the health of ICL; three-fifths of the capital investment is in ICL machines. The procedure for purchasing computers runs roughly as follows. For computers of power greater than one Atlas (the Atlas was a computer built in the 1960s; powers are measured by complicated assessments using benchmark programs) ICL may be invited to submit, singly, a tender. If, however, it is obvious that someone else can offer a cheaper and better machine over a shorter time scale the purchaser can go to open tender, but one of these tenders has to be from a British Company (which will be ICL). At the very large end (20 Atlas-power) ICL does not compete with the CDC 7600. But at 10 or so Atlas-power, the ICL 2980 in the 2900 range becomes competitive.

When ICL launched its new 2900 range in October 1974 it already had £21 million of orders, of which British universities had contributed £5 million. Ironically, when (shortly thereafter) the European Space Research Organisation (ESRO) ordered two 2980s they could only be supplied by persuading the universities to hold off for six months so that ICL could honour the ESRO contract.

Is the character of computing changing? In the past the regional centres were conceived as number-crunching establishments where immense calculations could simmer for hours on end. The number of scientists crunching numbers is not rising dramatically although, given half a chance, some of those who have contented themselves with one- and two-dimensional calculations would add an extra dimension. They are not likely to get that chance as the demand which is growing and which Flowers perceived in 1966 is for data manipulation. In such work the requirements are much more for easy accessibility of data bases, be they reference bases such as census results or dynamic bases such as meteorological conditions. There is little point in doing such operations down a telephone line, since the amount of data-shipping to interactive facilities may be colossal; the local centre comes very much into its own.

Ideally such data bases reside at the local computer, since they

Table 3 Equipment installed or on order at March 31, 1975

Manufacturer	Series (No. of machines)	Approximate capital investment (£k)
ICL	KDF9 (2)	490
	[formerly English Electric]	
	1900 (29)	21,140
	[formerly ICT]	
	4100 (11)	2,480
	[formerly Elliott]	
	System 4 (10)	6,080
IBM	[formerly English Electric]	
	2900 (3)	5,640
	Total	35,830
	System 360 (3)	2,130
	System 370 (3)	5,570
	Total	7,700
CDC	6000 (3)	4,680
	7000 (2)	4,460
	Cyber 72 (3)	1,580
	Total	10,720
DEC	PDP10 (1)	540
Burroughs	B1700/B5500/B6700 (3)	669
CTL	Satellite One/Modular One (39)	1,800

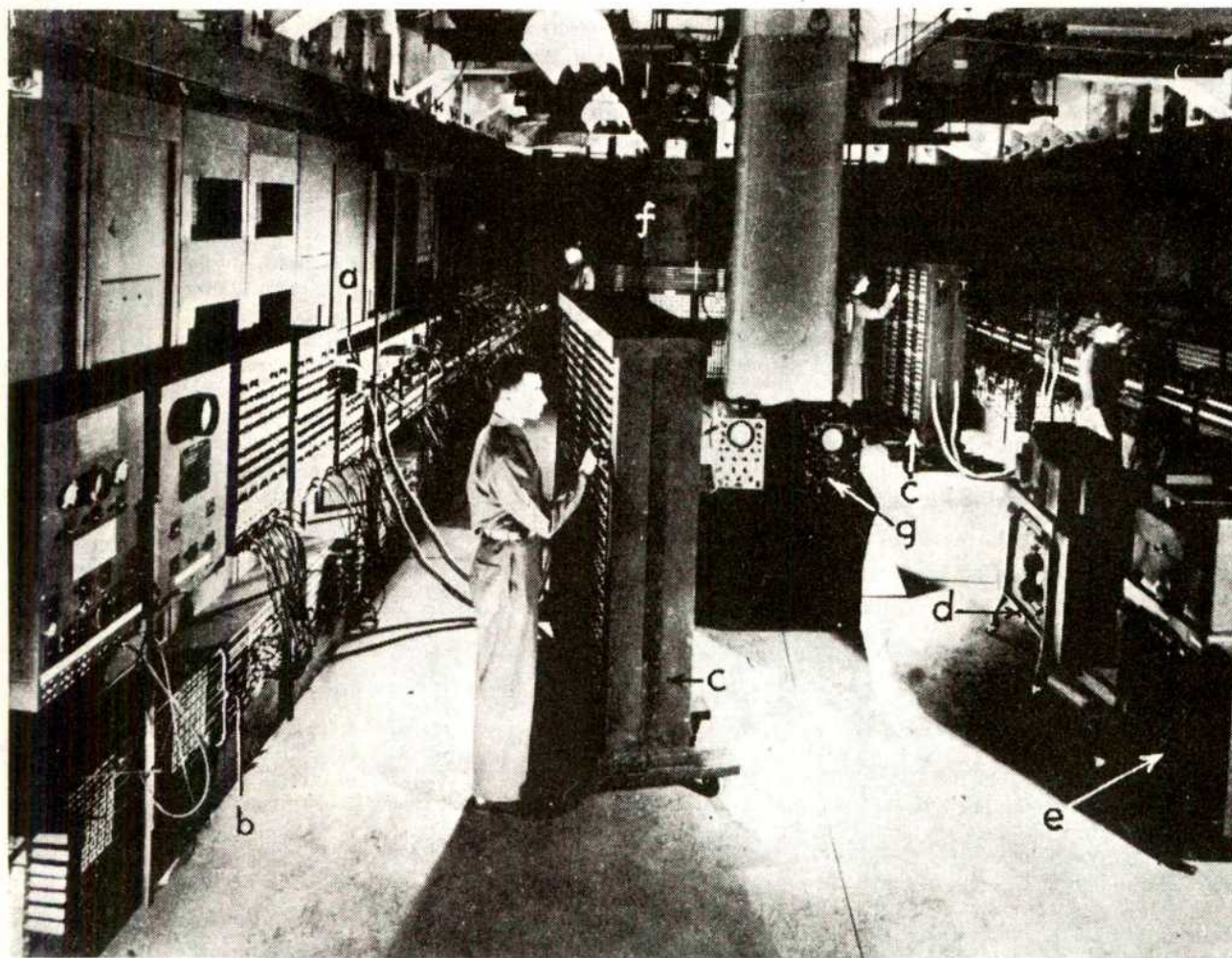
cost a lot to ship, but networking of a more comprehensive kind (such as Professor Kirstein describes in this supplement) is gradually becoming a serious alternative. The use of a network to distribute computing load is already explicit in the present phone-in arrangements to regional centres. The use of a network to handle a data base at a distance, or even a distributed data base in several parts of the country, requires further refinements; a scientist in Exeter must be able to handle a data base in Aberdeen by using programs resident in Aberdeen or even in East Anglia.

To this end several universities have expressed interest in using the Post Office's Experimental Packet Switching Service (EPSS) shortly to start operating and permitting data to be transferred at up to 48 kbits per second on a shared and sorted basis rather than by means of a continuous link. The eyes of the

more ardent data processors are bound also to turn towards the European Informatics Network, a project under the auspices of COST, the European Programme of Collaboration in Science and Technology. Ten nations are signatories to the project and at present five centres are to be joined by a packet switched network; the British link is through the National Physical Laboratory, Teddington. The network was not designed specifically for university use although universities in Milan and Zurich will be on it but it is possible that in the future it could be accessed through the EPSS to Teddington.

As far as bigger machines are concerned, there is little on the horizon. Sophisticated processors such as ILLIAC 4 or CDC Star 100 are not being thought of in a university context either in Britain or Europe, at least within the next five years.

The ENIAC, an electronic calculating machine



a, Digit trays; b, program trays; c, function unit; d, card reader; e, card punch; f, high speed multiplier; g, testing equipment.

The machine is built up in the form of a number of units each consisting of one or more vertical panels about 8 ft. high and 2 ft. wide, of which there are altogether forty. Each panel carries, at the back, racks of valves, relays, etc., and, at the front, switches, indicating lamps, plugs, sockets, etc. The different units are interconnected by two sets of lines, one set carrying signals expressing numerical information and the other set for control signals; connexions to and from these sets of lines can be plugged into the various units.

The whole machine comprises about 18,000 electronic valves, 3,000 indicating lamps, and 5,000 switches, and takes about 150 kW. in operation. Its flexibility and speed of operation will make it possible to carry out many numerical calculations, in many fields of investigation, which without its assistance would have been regarded as much too long and laborious to undertake.

Taken from D. R. Hartree's report in *Nature* (157, 527; April 20, 1946). See also *Nature*, 158, 500-506; October 12, 1946.

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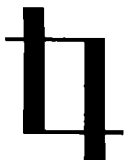


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The credibility of machine intelligence

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We usually use our previous experience in solving a present problem, and 'intelligence' is the name of the mechanism by which we do this. A programmed computer should not be deemed intelligent unless it is functionally similar to the brain in its use of previous experience.

WHEN Christopher Columbus sailed westwards in 1492 he hoped to reach India, and that is why the indigenous native Americans are called Indians. In 1975 there is an undercurrent of opinion that, in its established usage, the term artificial intelligence (AI) is as much a misnomer as Indian is a misnomer for the indigenous American. If AI is misnamed this is surely due to the difficulties of making a clear comparison between the problem-solving faculties of the brain and a computer.

To understand how the brain works, in the way that we know how an ordinary digital computer works, is perhaps one of the most important and urgent technical problems of the present time. The solution could provide scope for advanced automation that would very substantially alter and perhaps improve the present commercial and industrial scene. A mechanistic understanding of thought processes might to some extent benefit psychiatry. If you consult a heart specialist you can be assured that he knows fairly accurately how the heart works, but a man who consults a psychiatrist has no such assurance concerning the brain.

Science usually progresses by testing hypotheses or models. A computer that has been programmed to play chess, prove theorems, or understand natural language, may be claimed to model a thought process. To make scientific progress we should eventually compare such programs with natural thought processes (more completely than in protocol analysis¹).

Comparing computer circuitry and algorithms

In patent specifications of digital data processing apparatus the detailed physical implementation of 'and' gates and other common components is usually not specified. Instead, these components are usually specified merely as 'and' gates, 'or' gates and so on, and logical design diagrams are given which show how the various components are interconnected. For the purposes of patents, it may not matter whether the logical design of computer circuitry is implemented by transistor integrated circuits or by relay switching circuits. Thus, two computers may be deemed to be embodiments of the same invention even though, so to speak, their detailed physiology is very different.

By means of Boolean algebraic manipulation, a logical design can be changed to an equivalent logical design that looks superficially different. For instance a two-input 'and' gate can be replaced by a two-input 'or' gate that has its two inputs and its output inverted. Thus, if the logical design diagrams of two computer circuits are different, this does not necessarily mean that there is really an essential difference between them. Even if the logical designs were essentially somewhat different, this difference might be judged, for example in a legal action over a patent, to be so

slight that the two circuits should be deemed to be embodiments of the same invention.

It may be more difficult to make such judgments on algorithms that are more abstract and complicated than circuits usually are. Two algorithms are said to be structurally equivalent if, and only if, for any given set of input values the algorithms execute the same sequence of statements and follow the same sequence of branches². There are generally many essentially similar but structurally non-equivalent algorithms for computing any given function, just as there are equivalent alternative logical designs for the hardware that implements any given switching function.

Two algorithms are said to be functionally equivalent if, and only if, for any given set of input values they yield the same output values. For purposes such as proving correctness of algorithms, structural equivalence seems too tight a notion, functional equivalence seems too loose a notion, and some sort of compromise between structural and functional equivalence is required³.

Comparing brains with computers

In 1975 there is considerable knowledge of the physiology of nerve cells and the neuroanatomy of, for instance, cat, octopus, and man, but this does not provide us with a credible and generally accepted logical design for the brain or even for a part such as the visual system. We could, however, determine structural equivalence of two algorithms without using knowledge of any circuit diagram of any computer that could execute the algorithms, and absence of a circuit diagram of a brain should not therefore make us abandon hope of designing a machine that is in some useful sense equivalent to a brain.

A man may move a hand, or perform some other act, not in response to an external stimulus, but for some internal reason. A man may perceive an external stimulus but not take any physical action. Unlike many algorithms, therefore, a brain is not merely a device that determines an output corresponding to a given input, and this presents a difficulty in the determination of functional equivalence. In view of this we might confine our attention to the subset of behaviour that consists of making observable responses to stimuli, and determine whether there is functional equivalence between a brain and a model at least over this subset. There would be so many possible stimuli that we could not try them all in turn, and instead we might merely test that for a randomly chosen subset of stimuli the brain and the model made the same response.

Unfortunately this test would not establish functional equivalence, because it ignores the vital fact that a man's response to a stimulus generally does not depend on that stimulus alone, but also on his previous experience of stimuli and responses, and possibly on further rational and emotional factors. In other words, a man's output at any time may depend not only on his present input, but also on many past inputs and outputs. To be deemed functionally equivalent to a brain, a model should exhibit the same dependence of an output on many past inputs and outputs. This is particularly important if the model is intended to be intelligent, because natural intelligence is

concerned with using stored data derived from previous inputs and outputs to determine an output corresponding to a newly given input³. AI should surely model natural intelligence in this important respect, but the practical difficulties of determining this functional equivalence seem to be hopelessly great, because it is necessary to consider sets of previous stimulus-response pairs, and there are too many possible choices of the sets.

Nevertheless, it may be useful to pursue this discussion of functional equivalence a little further. A man's response to a stimulus may depend on previous experience, but not uniquely. That is to say, there are generally countless different histories of previous experience that will yield the same stimulus-response behaviour in a given situation. Conversely, a man may respond differently to the same stimulus on different occasions, without any significant change in his experience in the intervening time. In spite of the lack of a 1:1 correspondence between previous history and present stimulus-response behaviour, this behaviour generally depends on the application of intelligence to previous experience. To understand intelligence we should surely understand this dependence, and not confine our attention to present stimulus-response behaviour.

The solving of a problem, for example, proving a theorem, should for the purposes of this discussion be regarded as present stimulus-response behaviour. We usually use our previous experience in solving a present problem, and intelligence is the mechanism by which we do this. This is true even though there are usually countless different episodes of past experience that would serve us just as well. Intelligence is surely the mechanism by which we arrive at a procedure for solving a problem: intelligence is not the procedure itself. A model would be functionally equivalent to a brain only if the model used the same inputs of past experience as the brain and did not use any inputs that the brain did not use.

If a man copied from a text book a proof of a geometry theorem, we would not say in common parlance that he had used his intelligence in proving the theorem. If a man proved a theorem by executing a given theorem-proving algorithm manually, we would not say that he had used his intelligence in proving the theorem, because we would not be satisfied that he had intelligently made use of his own past experience. If a machine proves a theorem by executing an algorithm written by a man, this does not necessarily mean that the machine is intelligent, because from the vital point of view of inputs of past experience, the man and machine may not be functionally equivalent theorem provers, and indeed they may be radically and drastically different theorem provers.

Even if the functional equivalence of past experience to a brain could be determined, it would not provide a satisfactory criterion for intelligence of a machine, because a brain has other things going on in it (instinct for example) besides intelligence. Furthermore, intelligence has many different levels and different manifestations⁴. It is possible that a whole class of algorithms, not just one, should properly be called intelligent. Many somewhat different algorithms are properly classified as top-down parsing algorithms. Many somewhat different algorithms are properly classified as linear programming algorithms. To be classified as a linear programming algorithm, an algorithm need not be functionally equivalent to any given linear programming algorithm. An algorithm might be properly classified as intelligent even if it were not functionally equivalent to any given intelligent algorithm or to any brain.

Exact functional equivalence seems too strong a criterion, so we must fall back on a looser notion of functional similarity. It would surely be wrong to say that a model was intelligent unless it was functionally similar to

an intelligent brain in its use of previous experience in problem solving.

Not comparing brains with computers

McCarthy says that the main scientific activity of AI researchers is, or should be, "studying the structure of information and the structure of problem solving processes independently of its realisation in animals or humans"⁵. McCarthy's statement broadly defines an area within the field of computer science, and if only some name other than artificial intelligence had been assigned to this area, there would be less confusion and misunderstanding. Just as one is not free to define an artificial diamond to be a lump of coal, so also one is not free to assign the name 'artificial intelligence' to a field of study that may only be obscurely related to intelligence. All such relationships are likely to remain obscure until intelligence is precisely, scientifically, and acceptably defined.

But by clarifying judiciously chosen computational problems, and by discovering and comparing different methods by which these problems can be solved, we may perhaps eventually begin to agree that some problem solving processes are more intelligent than others. It is possible that two solutions to a given problem may be equally successful from a pragmatic point of view, but one solution may be judged more intelligent than the other in view of the type of past-experience data that it uses.

The use of computers in weather forecasting and in the computation of rocket trajectories involves machine representation of knowledge but does not belong to the AI field, presumably because the computations are numerical. AI^{6,7} is concerned primarily with those non-numerical problems, such as chess, theorem proving, and robot control, at which men excel over computers. In solving such problems there are two schools of thought in AI, the generalist and the specialist⁸. The specialist school is prepared to make more use of *ad hoc* programming than the generalist school.

To make a computer exhibit limited understanding of natural language^{9,10} it is convenient for the programmer to use his knowledge of the world, that is, his semantic knowledge, in writing procedures, and this practice is called procedural embedding of knowledge. This practice is characteristic of the specialist school⁸. When new knowledge is to be used, new *ad hoc* instructions must be written, and considerable reprogramming may be required when a procedural embedding system is extended to cope with a somewhat new problem domain¹¹.

The Stanford Research Institute problem solver is used to control a mobile robot in tasks such as finding a box and pushing it through a doorway into another room¹². The core of the robot control system is an automatic theorem-prover that determines plans of action by proving theorems in predicate calculus. Knowledge of the world is expressed in statements in the predicate calculus, or, in other words, knowledge is assertationally embedded in the system. Assertional embedding has the advantage that the problem-solving system can be extended to new problem-solving domains without extensive reprogramming, since the principal changes can be made merely by changing predicate calculus statements. The theorem prover is a general purpose program that is independent of specific problem semantics, and this¹¹ provides us with a very clear example of generalist work. It is less clear how much generality there is in the interface between the real physical world and the assertional representation of it. In a generalist system the requirement for generality precludes the limitless sophistication that can be built into a specialist system *ad hoc* programming; and the problem of keeping abreast of changes in the world may be particularly acute in a generalist system¹².

Acquisition of knowledge

Whether knowledge is represented procedurally or assertionally, it must first somehow be acquired. Feldman and Yakimovsky¹³ have devised a system that automatically labels area of a photograph as "sky", "mountain", "grass", and so on. Indeed the system partitions a photograph into such areas. This system uses previous knowledge that in the world "sky" is above "mountain". Here, "above" is a relationship, and in partitioning a photograph the system exploits knowledge of many such relationships: Feldman and Yakimovsky have developed a general technique for using such relationships, provided that these are given to the system. The system has statistical learning capabilities but has no means of automatically acquiring knowledge of these relationships, for instance by processing unpartitioned specimen photographs of many different scenes.

This acquisition problem does not seem to be intractable, and indeed I have already investigated a related problem¹⁴, the first of a series in AI¹⁵. Although this work was admittedly abstract, primitive, and impractical, it did show that one can make headway with the segmentation of a pattern into parts, given as data only a set of unsegmented specimen patterns composed of different combinations of parts. A solution to this problem would seem, to my intuition, to be nearer to intelligence than would a segmentation technique that relied on a human to supply knowledge of relationships between parts. This also serves to illustrate my previous remarks about previous-experience input data.

For practical purposes of segmenting a photograph into semantically homogeneous areas (for example in aerial photointerpretation) there may be no advantage in automatic acquisition of relational knowledge that is used for achieving segmentation. Instead, this problem of acquisition may be left to those of us who do not forget that there is only limited information capacity in the genetic code by which the brain structure of an offspring is determined by a parent, and to those of us who would prefer to model the lower levels of practical intelligence⁴ before proceeding to the understanding of natural language by the machine^{8,16}.

Intelligence

In a top-down parsing algorithm, or any other kind of algorithm for that matter, there is no single instruction that is the "heart" of the algorithm. A top-down parsing algorithm is recognisable as such by its overall structure, in spite of the fact that it contains no tangible heart. A written word is recognisable as a combination of letters, despite the fact that it contains no letter that other words

do not contain. An algorithm that should properly be called intelligent may be recognisable by its overall structure, and "we should not let our inability to discern a locus of intelligence lead us to conclude that programmed computers therefore cannot think"¹⁷, but this does not imply that all problem solving algorithms should properly be called intelligent. The presence or absence of a heart, locus, or "blinding white light" is irrelevant to the determination of intelligence of an algorithm.

To Minsky "... 'intelligence' seems to denote little more than the complex of performances which we happen to respect but do not understand"¹⁷. To me "intelligence" denotes a very distinctive class of problem-solving processes, and I do not think that the word "intelligence" should be applied to radically different classes of problem-solving processes, even when these successfully solve the same problems.

Finally I shall hazard some guesses about the distinctive nature of intelligence. It seems safe to guess that, in the brain, intelligence is realised by a highly parallel computation using distributed logic. This computation could presumably be simulated on an ordinary digital computer, but perhaps awkwardly, inelegantly, and even impractically. Animals and people do not always seem to need the same sort of instructions that computers need, and I guess that in some cases intelligence is realised in the brain by a sequential process that does not use stored instructions: the process is non-programmed, not self-programmed, in that there is no stored program. I guess that in the design of the brain there are solutions to two of the salient problems of computer science: the mitigation of programming effort, and the organisation of parallel computation.

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Distributed computer networks

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How desirable and inevitable are large heterogeneous, distributed computer networks? In this article Professor Kirstein, whose group at University College London operates one node of the large international ARPA network, describes the motivation for setting up such networks and the problems which must be resolved before they are deployed more widely. Those problems are often more political and sociological than technical.

THERE is considerable argument as to what is meant by a computer network. Any large modern computer system contains several processors and for commercial or technical reasons these may be mounted in one set of cabinets. Nonetheless, they could still be regarded as a network, and here I shall define a computer network as a collection of computers, connected through communication lines to other computers or terminals. This definition will not please all the proponents of computer networks: some will consider it too narrow, because it does not include single collections of connected

processors; others will consider it too wide because it includes Star systems (Fig. 1). There is, however, a qualitative difference between the purpose, technology and potential of the systems which come under my definition of a computer network and single collections of connected processors.

Figure 1 shows the principle ingredients of a Star-organised computer network. A single computer centre (C) is connected through a data network (N) to a number of terminals (T). Each of the elements C, N, and T can have different levels of sophistication. The computer centre can be one processor or a set of computers at one site (perhaps interconnected, as shown in Fig. 1). The data network can be just the public switched telephone network (PSTN), the British Post Office experimental packet switched service (EPSS), a distribution system based on leased lines and privately owned switching computers, a set of leased multiplexed, telephone channels, or a mixture of elements like these. As an example, Fig. 1 shows a hierarchical leased network of central concentrators (CC), and remote concentrators (RC) connected to the PSTN. The terminals may be either simple keyboard terminals (or displays), or more complex computer work stations which control card readers, printers, magnetic disks and tapes as well as keyboard terminals. A key aspect of all these computer networks is the desire to increase the catchment area of the individual computer sites, and to make the computers available to people elsewhere.

In a very simple system, the C of Fig. 1 may be only a single computer, the N, the PSTN, and the T keyboard terminals; many T of the smaller time sharing bureaux are so configured. A single computer (H in Fig. 1) is not, however, sufficiently reliable; for better availability, multiple computers are incorporated in the computer centre. The PSTN is adequate for local access to keyboard terminals. To provide a larger catchment area at cheaper cost, however, the installation of concentrators (RC in Fig. 1) connected by leased lines to the computer site is more cost effective. The PSTN is restricted, in the UK, to 300 bits per second (b.p.s.), which is 30 characters per second in two directions simultaneously, or somewhat higher speeds asymmetrically (for example, 100–200 characters per second one way, 7 characters per second the other). Thus, to provide higher speeds (for example, for batch card reader–line printer terminals) leased line networks are requested. These lines can go direct to the central site, C, or to nearer concentrators (RC in Fig. 1).

Because the computer systems themselves were the least reliable components, it was unnecessary with the earlier networks based on single computers in central sites to put much sophistication in the data network. Such systems are still limited, however, to serving about 100 users simultaneously. To improve availability, multiple mainframes were incorporated in C. Moreover, performance was improved by introducing a certain amount of functional specialisation. For example, in both the London¹ and Manchester University regional computing centres, one machine is being used for the main large batch processing, and other front-end computers (FE) deal with the interactive traffic and submit work to the batch processor.

No commercial limit has been reached yet on the number of terminals that can be serviced simultaneously by a system with the topology of Fig. 1. Indeed, some of the larger time sharing bureaux have used it to service several thousand terminals simultaneously. Of course, in those circumstances the organisation of C is complex, with duplicate access to the communication lines, processors and files. These precautions guarantee the continued availability of resources in the event of failure of most individual subsystems.

The data networks are configured very carefully, with due consideration given to the connections of RCs to CCs, duplicate CCs, the optimum siting of RCs, and so on. Such systems must provide highly reliable access at least for interactive activities. The General Electric Network² and TYMNET³ are the best developed of this class.

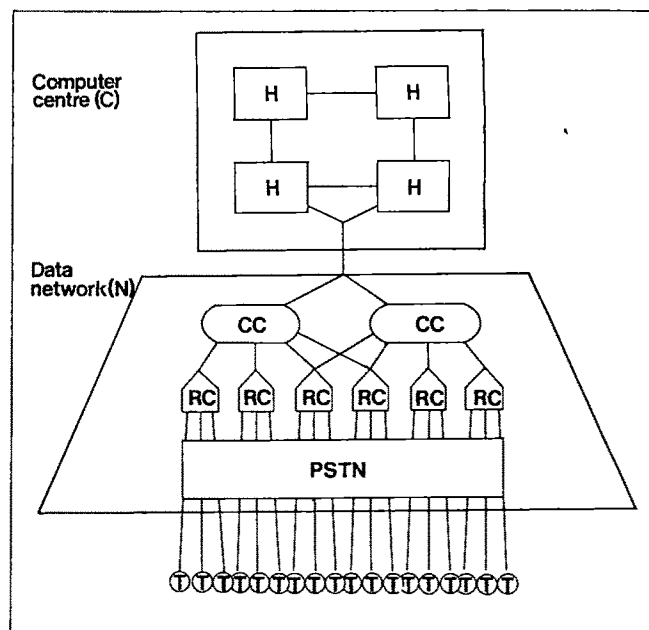


Fig. 1 Star-organised computer network. H, Hosts; CC, central concentrators; RC, remote concentrators; PSTN, public switched telephone network; T, terminals.

The availability of different processors in C (Fig. 1) allows a considerable degree of functional specialisation. One or more systems may be used only for interactive editing, others for access to specialised data banks, and yet others for very large parallel processes. One noteworthy example of this functional specialisation is the ILLIAC IV complex at NASA-AMES⁴. At last count it contained more than 30 computers, from those driving the 10^{12} bit store, controlling the communication functions, to those providing interactive file manipulation and controlling the 64 parallel arrays or processors.

There is one facility not provided in the systems of Fig. 1: geographical separation of the host (that is, the main service machine). For many reasons this distribution can be very important, geographically distributed sites may have a large local catchment area, so that only a small proportion of computing need, or should, be exported. A balance between gains from larger computing centres against added communication costs can make the topology of Fig. 2 most attractive. In practice, computing centres are often brought into being for local reasons, but cannot supply all local needs. In such conditions the topology of Fig. 2 is a natural outgrowth. Here, the individual host sites, C, may have the form of Fig. 1 in its simple or more complex embodiments, each site responsible for a distribution network, N, to its own customers, T. In addition, there is now a high level data network (HLDN) connecting the host sites. The terminals can be switched through the HLDN to other sites. Three networks which use this technique are the early versions of ARPANET⁵, the French Cyclades⁶ and the UK South-west Universities Network (ICNS)⁷. As a final level of sophistication, the terminals (T, in Fig. 2) can also be introduced directly to the HLDN, as on the present versions of ARPANET⁵ and TYMNET³. Obviously, practical networks do not always allow a neat classification into those of Fig. 1 or Fig. 2. For example, the General Electric network² is essentially of the type of Fig. 1, with a high level data network connecting individual computers in the computer site. The computers themselves are distributed in two centres, and could be spread over more than one network with no change of technology.

Using the networks shown in Fig. 2 it is possible to have a more rational growth policy. One centre can be expanded and some of its surplus used to delay the need of expansion at other sites. Centres not willing to forego their own computer systems may be much more willing to supply a highly specia-

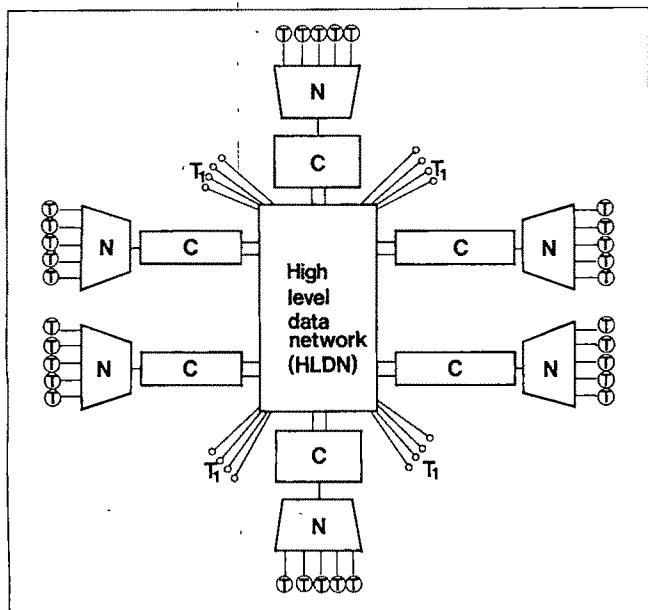


Fig. 2 Distributed computer network. HLDN, High level data network; other symbols as in Fig. 1.

lised proportion of the computing requirements for a whole community. The difficulties posed are not technical but political and sociological.

The national communication carriers would like to offer the maximum portion of data network traffic; at the least, they will always lease transmission channels. Many are proposing special data networks which also provide any switching and multiplexing required. Examples of planned offerings in this area are the US TELENET⁸, the UK EPSS⁹, the French TRANSPAC¹⁰, the German EDS¹¹, and the Nordic network¹². The communications carriers would like to separate out the data network portion as much as possible from the rest of the network, and some of the commercial interests (for example, TELENET and TYMSHARE) would like to provide them as commercial services.

With the systems of Fig. 2, very careful definition is required for the communication protocols at different levels. When the different computer systems are from the same manufacturers, as, for example, in the South-west Universities' network, the problems are easier. But when the systems are different, as in ARPANET⁶ and Cyclades⁶, very careful design and implementation is required¹³. Sometimes, as in the UK EPSS, the carrier defines one portion of these protocols, but in such

cases it may still be necessary for those responsible for connecting in computer sites or terminals to define other levels¹⁴.

The data communication network may be very heterogeneous itself. Besides containing a very hierarchical structure, it will in the future include new technologies. In the US ARPA has been used to investigate the use of satellites for broadcasts¹⁵.

A set of experiments to investigate this technique, to be carried out jointly by ARPA, the British Post Office and a number of research organisations (including my group at University College, London (UCL)) started in the summer of 1975. ARPA has also been used to investigate access to the local nodes by packet radio¹⁸.

The ARPA computer network and UK usage

In the ARPA network, the sites are connected together by a communication subnetwork (ARPANET), 50×10^3 b.p.s. (Fig. 3). This comprises leased lines (usually at 50×10^3 b.p.s.) connecting communications computers called Interface Message Processors (IMP)¹⁷. The system has extra connections to permit alternate routing in case one node becomes inoperative. The host computers (H) are connected to the IMPs, and may be local or remote; in the latter case the connection between the IMP and the host is by a leased telephone line. In some cases only host computers are attached to the IMP; in others, terminals can also be attached. The latter set of communication computers are called terminal interface message processors (TIPs)¹⁸.

The important attribute of this network is that messages between hosts are split up into packets. Each packet is up to 1,000 bits long and contains the full information about its source and destination in its header. The IMPs contain the information on how to route from the source to the destination. Cost saving arises from the possibility of using a much smaller number of communication lines than would be required in a system in which each pair of nodes was joined by a pair of lines. The fact that the destination information is contained in the header saves the time and overheads required in setting up a virtual circuit. It is possible to use high-bandwidth lines with appropriate cost savings, and achieve a maximum transmit time between sites, in the absence of satellite hops, of 200 ms for single packets of 1,000 bits. ARPANET now stretches to Hawaii, Norway and London (Figs 3 and 4).

The connection of those sites sited near the IMPs to ARPANET relies on a special hardware interface because of the lack of standard communication interfaces. This policy has also been used in later developments of ARPANET and similar sub-networks^{5,8}. A considerable body of software is required in the hosts if this heterogeneous collection of computers is to be used in a somewhat homogeneous manner. There is a network control program, which embodies the procedures agreed for a process in one host to connect to a process in another. There

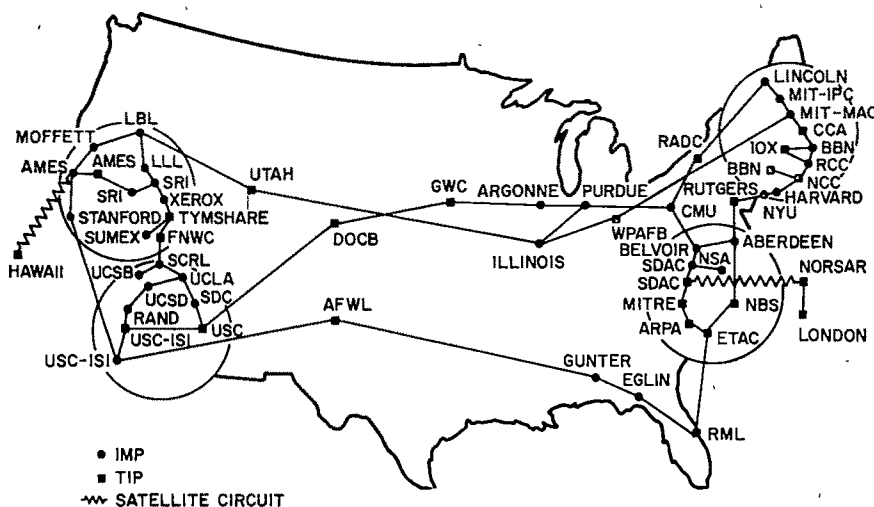


Fig. 3 Geographical map of the ARPA network (April 1975).

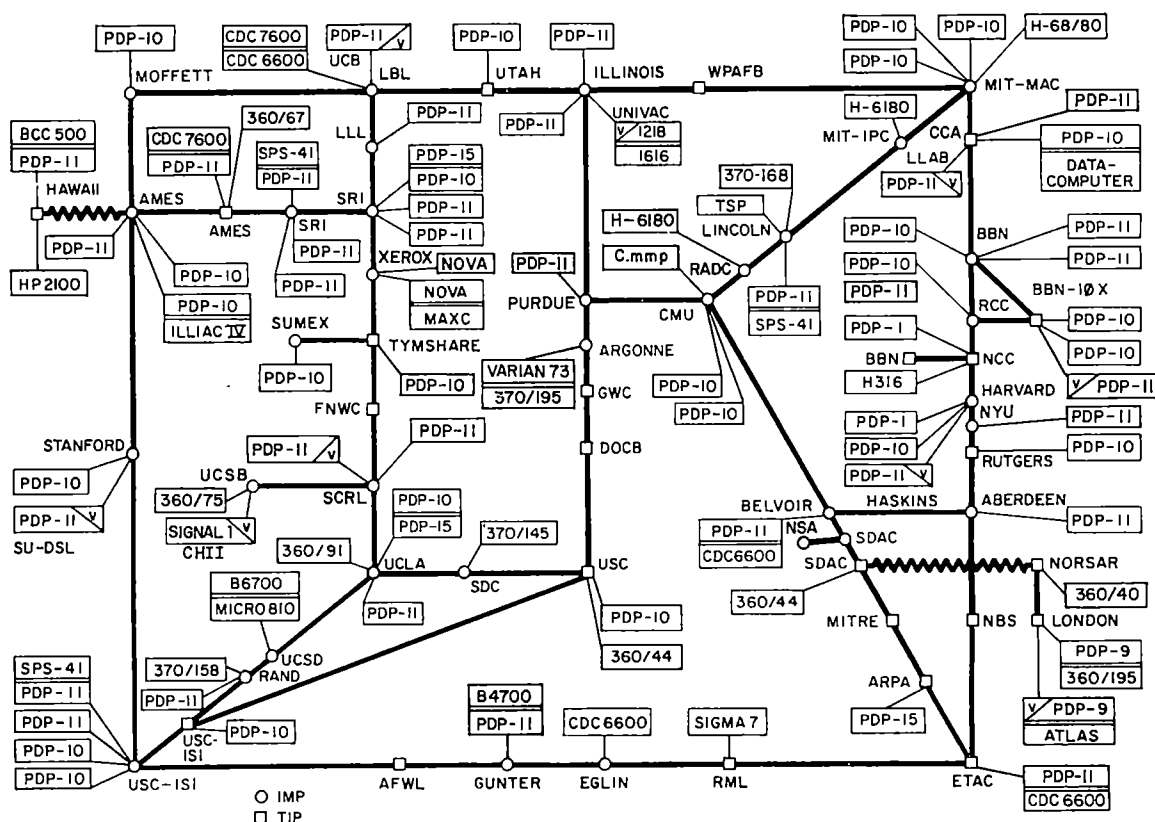


Fig. 4 Logical map of the ARPA network (April 1975).

are protocols for network virtual terminals and file transfer. Even higher functions such as Graphics and Mail Transfer have been standardised¹³. In this context the word 'protocol' means an agreement by the participating sites to implement programs which will accept the standard format of data and control for the relevant functions.

The ARPA network has not gone as far as possible in standardisation. Though there have been discussions of network-wide job control or command languages, and even editors, these have not been implemented in practice. This was partly because of the lack of effort available, and partly because of the increasing difficulty in agreeing on standards for the higher functions. In our experience about 8,000 words of code (in our system 4,000 words resident) are required to implement these protocols.

To operate such a network it is essential to have a network control centre¹⁹. Extensive information on how to use the systems must be made available. Much of this is kept on-line on the host computers; however, a special facility, the Network Information Centre, has been set up for this purpose. Lack of funding has not allowed this to be kept up properly leading to some problems. In the UK, my group is providing a documentation and advisory service to other UK groups using the network. For this purpose we have arranged to make many documents available in a special collection in the British Library (Lending Division).

It is very difficult to organise efficient advisory and co-ordination activities in such a network without good, simple message services. For the research most of the hosts have instituted some facilities for exchanging text messages between the users. The procedures have been standardised, so that the messages can be sent to other sites. Simple composition and retrieval facilities have also been provided (for example, simple editing, ability to append lists of addresses, single line titles, procedures for listing only the titles of messages, and so on). In addition, there are facilities for linking terminals together to enable two or more terminals to communicate in

real time with each other—even though they are attached to different hosts. This is very interesting and complex²⁰, though its uncontrolled use would be against the regulations of the PTTs. In the context of the international ARPA network its use is only permitted for specific purposes concerned with the computations in progress. An important lesson to be learned from the ARPA network is that the communication sub-network, ARPANET, which is the entity which will in future be provided more by the PTTs (refs 8–12), is only one part of the infrastructure required to make such networks function effectively. The subnet, because it is usually implemented by one organisation, should proceed smoothly. The development of the rest of the infrastructure can be much more time consuming.

In the context of scientific multi-user networks the types of use which are being made of the hosts will be of interest. The ARPA network is being used operationally for a large number of projects. It is the principal means of entering jobs into the Illiac IV computer; a substantial proportion of the use of MULTICS, the UCLA IBM 360/91, and many of the TENEX sites work through this network. Among the substantial applications of ARPANET are the acquisition and analysis of seismic data, large scale climatic modelling, the administration of ARPA projects, and the coordination and cooperation of hundreds of research programs. A partial list of projects which used the UCL link of the ARPA network during 1974 is given in Table 1. This list encompasses a broad range of subject areas, research institutions, and government departments; the uses are discussed much more fully elsewhere²¹. Most, however, involve cooperative activities, between different research groups in the UK and USA. Sometimes the cooperation involves shared use and development of the same programs (for example, University College and the Royal College of Arts, Table 1); sometimes the computer resources of one group are traded for those of another; sometimes one group has access to a computer system belonging to another so they can develop software of mutual interest

(for example, Utah's use of the RL 360/195). Sometimes the incentive to cooperation is access to data bases built up jointly or just accessed by more than one group (for example, the Blacknest Seismic Data Base and the British Library use of Medline)²², sometimes the main motivation is just to have the groups share their research experiences more promptly and completely (St Bartholomew's Hospital and Southampton University's use of the teleconferencing system Forum)²³. Of great importance here is the unpredictability of the uses. In 1971, when the UCL project was proposed officially, there were eight proposed users (all in computer science); of these only half ended up using the link in practice. All the others in Table 1 starting applying (and are still applying at the rate of about two a month) once the link was established.

Motivation for computer networks and their development

There are six fundamental reasons for preferring the use of a network to that of a local machine.

First, the cost of running a particular job may be substantially lower on a larger machine than on a smaller machine, even allowing for communication costs. For that reason, a larger computer than is required for local computing, with a suitable data network to enlarge the catchment area, may be economically attractive. That is the rationale behind the establishment of the University Regional Computer Centres by the UK Computer Board. Wells²⁴ has commented that the cost of processing goes down as the square of the computer power, whereas the cost of file storage varies linearly for any particular machine type.

Second, with a large collection of computers in a network and corresponding catchment area, it is possible to make smoother increases of capacity to meet demand, and to provide better reliability and availability. This is one of the rationales behind the TYMNET and General Electric networks.

Third, with a large catchment area, covering many time zones, the diurnal demand cycle means that the peak computing requirements of some of the users will come at the low activity periods of others. The effect is very pronounced in

the networks which span North America, Europe, and the Far East. Even with Europe and North America covered alone, the effective peak day is 18 h long.

Fourth, specialised resources like data bases, programs, or special hardware, which would otherwise not be available, can be accessed. This is the major motivation of many of the users of service bureaux and stock exchange dealings also come into this category. In the scientific field seismic and weather data bases, bibliographic data bases, large data bases developed for high energy physics experiments and molecular structure come into this category.

Fifth, for research or other groups to cooperate on the same or related projects they may need access to each other's data or programs. They may also need to share messages with short response times, or even in real time, with several other people at different sites.

Finally, there may be a requirement of computing capacity at each site; the network environment enables one site to increase capacity beyond the local requirement and export some capacity to others in the network. This leapfrogging technique enables all the sites to vary their systems progressively with minimum dislocation and short term over-capacity. It enables different centres to specialise on specific areas, while keeping all areas available to local users. The South-West Universities Network works like this.

For some applications the type of network used is immaterial, but traffic considerations and the cost of communications make those like the ARPA network the most economic. This is particularly the case when there is a genuine need for local computing—often resulting partly from the difficulty in attracting good staff without a local computer.

A closer look at the costs of the different applications of computers, shows that there are often considerable advantages in dedicating computer systems for specific applications or types of service. One reason for this is the pure hardware and software cost of providing the computation for each item. Another is to avoid the cost of duplication at development and storage stages of the packages, and to provide the relevant advisory facilities.

Table 1 Partial list of UK project using the ARPA computer network through the UCL node (March–December 1974)

UK organisation	US partner	Purpose
Blacknest Research Establishment	Lincoln Laboratory, MIT; NORSAR, Norway	Cooperative construction and analysis of seismic data bases
British Library (Research Division) with 15 UK centres under their sponsorship for a specific experiment	National Library of Medicine	Experimental comparison of MEDLINE, a medical bibliographic information retrieval system, with a similar UK system
Cambridge University	Utah University	Development and comparison of algebraic manipulation system
Culham Laboratory	NASA-AMES	Application of ILLIAC IV to magneto-hydrodynamic computations using symbolic manipulation techniques
Royal College of Art	Harvard, MIT	Development and evaluation of space-planning systems in architectural design
Rutherford Laboratory (including its user groups at Oxford and Surrey Universities)	Illinois University Maryland University Harvard University Chicago University	Exchange of software and data for theoretical high energy physics calculations
St Bartholomew's Hospital Medical School	Brookhaven National Laboratories	Teleconference on human response to sulphur pollution
Southampton University Politics Department	University of California, Santa Barbara	Teleconference on political simulation models
University College London, Department of Statistics and Computer Science	Stanford University Bolt, Beranek and Newman	Experiment on the requirements for interconnection of computer networks

A rational policy of computer provision for, for example, the university and research communities would therefore include some local, general purpose, computing facilities (probably not connected to a national network), some regional, large scale, general purpose computing facilities (part of a regional network), and some specialised facilities (clearly, part of a national, and in some cases an international, network). Some types of computation may be done best in commercial bureaux, and even that should be permissible. Together with the build up of computer facilities in this way, some control is essential to ensure that the computing is done on an appropriate facility—and that the operators of facilities do not duplicate unduly activities already carried out well elsewhere. Such a computer policy raises many problems: on the one hand it is ludicrous to develop a complete expensive body of programs and data bases in a specific area as a general service, if another adequate set exists already accessible by the same user community; on the other, one should not prevent progress in providing new and better facilities. Sometimes, such improvements can be made in an evolutionary fashion but at others a completely new system is required.

Using any network on a large scale requires resolution of 'balance of payment' issues. It seems a fact of life at the moment that institutions want the maximum facilities locally, and resist resources going elsewhere. They prefer to be net exporters—and to be paid to export. For the concept of specialised resources to work, means must be found for financing their development and utilisation—even at the cost of local generalised facilities. Resolution of this problem becomes progressively more difficult as the computers become wider apart.

In the UK, the Science Research Council has succeeded in this respect with the High Energy Physics Community. Regional University computing centres have been installed for large scale general computers. Only now is there discussion of a 'computer aided design' centre for the universities (though there is one in Cambridge run by the Department of Industry). This concept should be carried much further—but needs a widespread data network to permit its implementation. A candidate for this network should be in existence soon in the UK with the development of the Post Office's Experimental Packet Switched Service⁹. Technically, international usage is possible, and there are communities who desire and require such use; it must be resolved, however, whether the appropriate payments or regulation mechanisms can be devised.

There is a further serious question to be resolved on network usage, and that is a complex mixture of regulatory and economic issues. No tariffs for EPSS have been published. It

is clearly cheaper to set up private user data networks (like those of Figs 1 and 2) than to use only the facilities provided by the Post Office—though this is not necessarily so in the US where 'value added networks' are permitted²⁰. In the UK, and even more internationally, there are severe restrictions on which users may join together in a private network. There are, for example, several very large bibliographic data bases owned and operated by different organisations²². These fall clearly into the description of the 'specialised resources' mentioned already. In the UK (and in many other countries) a private data network operated by one organisation is not permitted to provide the public access to several such systems.

This problem is even more difficult if the common user service is to be provided internationally. We can expect a continued dialogue between the PTTs and the service suppliers and user communities to modify tariff and regulatory policies in the light of economic pressures and technical advances.

Technology now exists for large scale, distributed, heterogeneous computer networks. The most common method of implementing them is by a separate data network with host computers attached. There is still some discussion as to what should provide the data networks, what facilities they should contain, and how they should be tariffed. The main bar to a wider deployment of such networks is the need to resolve the organisational, regulatory and financial issues they raise.

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Computers and the printing industry

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As page composition from digitised information replaces the manual assembly of type, the printing industry struggles to absorb a technology in which the compositor becomes a computer operator and the production controller a systems analyst.

CRITICS of print technology are fond of pointing out that if Caxton returned to the industry today he could walk back into his old job with the help of a fortnight's refresher course at the London College of Printing. Certainly it is true that

Gutenberg, who developed moveable type in the fifteenth century, would have no great difficulty today in locating a composing room which used materials and crafts recognisable to him, whether in his native Strasbourg, in Britain or the USA.

For many years it seemed strange to technicians outside the print industry that something like an office typewriter could not be put on-line to a computer and thence to the printing process. In 1867 a Milwaukee editor, Christopher L. Sholes, patented the machine which gunmakers E. Remington and Sons manufactured as the Remington Model 1, yet a century later even the simplistic use of an IBM typewriter driven by punched tape or on-line to a small digital computer is an innovation in some print houses.

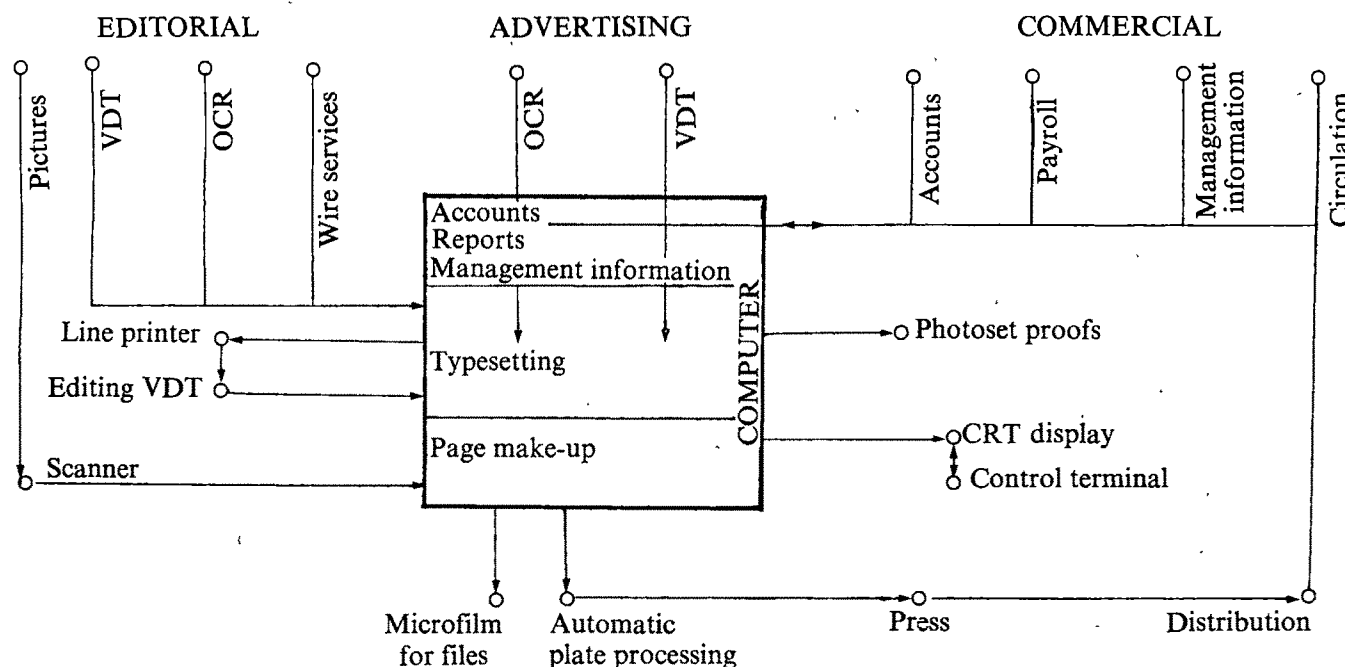


Fig. 1 A theoretical typesetting and page make-up system for a daily newspaper. Picture input is the present obstacle.

Countless frustrated authors and publishers have condemned the mediaeval technology of typesetting while at the same time feeling some affection for the traditional techniques which for centuries defied any degree of mechanisation. It is therefore in some ways sad but probably inevitable that the computer in print is ultimately destined not to refine the traditional craft skills but rather to replace them.

If printing is considered a two-part operation comprising first, the processing of words into a printable form and, second, the manufacture of a readable product (which is possibly in the long term an unnecessary breakdown), then the first part at least can be achieved at present by computer-driven hardware controlled by technicians who are not necessarily trained printers.

The feasibility of the jump—after 500 years of technological stagnation—from techniques which involve the individual collation by hand of lead letters or lines to systems which effectively dispense with manual skills altogether can only be appreciated in the context of the second part of the production process, the printing operation, since this largely governs the form of origination necessary.

Caxton and Gutenberg, on their reincarnation as twentieth century printers, would print by the letterpress process, involving the pressure of an inked relief image against the paper. In this case, the opportunity for the use of a computer to progress beyond mathematical problems of letter assembly (such as word and letter spacing) is qualified by the unavoidable complexity and size of the machine which would then be necessary to assemble automatically a page of relief metal. Consequently only limited mechanical assistance of any sort has proved to be economic in metal typesetting. It is possible and usual, of course, to cast type in 'hot metal' by machine, but normally this process ends with the collation of lines of type, after which pages have to be made up skilfully by hand.

The Monotype, which automatically casts individual letters in lead on instructions from a keyboard perforated paper roll was invented by Tolbert Lanston in 1887; Mergenthaler's Linotype, which casts lines of lead type in a semi-automatic process from directly keyboarded commands, went into production a year earlier and became the standard newspaper setting machine. Still in general use as updated models throughout the world, both machines retain the appearance of having been created by Heath Robinson after a heavy night out.

The Linotype and Monotype represent the most commonly used methods of mechanically casting metal type but manual expertise is needed both in operating complex keyboards and in making up pages from the set type; multiple installations are necessary if any volume of setting is to be achieved.

Computers are successfully used to produce perforated tape to drive linecasting machines, but the metal output is too restricted to allow much application beyond the production of lines of justified type.

Although letterpress has inhibited computer potential, the commercial development of offset lithography has encouraged it. The offset lithographic printing process was discovered accidentally in 1796 by Alois Senefelder who was working on a method for reproducing music notation, but it was not until well into the twentieth century that extensive commercial use was made of the process. Lithography uses a planographic plate which is developed photographically, so anything which can be photographed—even typewriting, for example—can be converted into a printing plate.

The lithographic technique involves the use of pre-sensitised plates exposed through a film (negative or positive) of the page of type and illustrations. Development of the exposed plate leaves the non-printing areas hydrophilic with the printing surfaces ink-attractive. Judicious application of water, then ink, to the cylindrically mounted plate allows the transfer of the exposed, inked area to a revolving rubber blanket and thence to the paper.

Replacing metal

As offset lithography slowly replaced some letterpress installations, the inconvenience of metal type became increasingly burdensome. Since input to the lithographic plate-making process was in the form of 'flat artwork' ready to photograph, metal type needed proofing on high quality paper before it was ready for the camera.

Alternatively, other methods of composing type could now be used, and as paper or film—as opposed to metal—output was required from the composition process, electronically controlled typesetting machines became convenient and economic.

Two main systems have been used extensively: a 'near print' process which uses a typewriter-based device such as the Friden Justewriter or IBM Selectric to 'type' setting as a straight

manual operation or from coded tape, and a photographic process in which the typesetting is produced as film or a bromide print. Both methods can use binary logic to solve the arithmetical problems involved in setting letters of varying widths, but for various reasons, including speed, versatility and the suitability of the end product, photo- and filmsetting have made the widest use of the computer. It is therefore relevant to look briefly at the mechanics of photosetting.

The early filmsetters (for example the first Monophoto) were mechanically based on hot metal casters with matrix cases of typographical characters on film replacing the caster's case of 'moulds'. A quartz-iodine light source projected a selected character on command on to photographic film. Most electronic photosetters, on the other hand, store characters on disks, drums or grids. Disk or drum stores rotate continuously and characters are illuminated by xenon flash tubes. Grid characters are exposed in a stationary position. The sizes of characters may be controlled optically by moving or changing lenses within the exposure routine or by storing character negatives in varying sizes.

Such photosetters may be quite sophisticated in themselves, with special-purpose minicomputers controlling the word and letter spacing, or they may be 'slave' units operating on instructions from a separate computer. Input is usually by Qwerty keyboard, either direct entry or off-line, using punched tape. If a small photosetter is using its own minicomputer, keyboards will normally be off-line, since its output speed will permit the consecutive acceptance of tapes from several keyboards while its computer will not have the power to store or process several inputs concurrently.

The fastest photosetters, mainly evolved in the past three years, use a cathode ray tube (CRT) to recall digitised characters which can be kept on file in a backing store. CRT photosetters are very fast and under intensive development, and are rapidly becoming economically viable for medium-sized printers.

Justification routine

The extent to which computer power is used in typesetting depends on economics, speed, volume of work, complexity of material processed and the number of ancillary operations involved.

Justification was the first and most obvious task to set a computer. By tradition, most printed matter, unlike typewriting, has proportionately spaced characters (that is, letters of varying width) and a straight (or 'justified') right-hand margin. The justification is achieved by varying the spaces between words within set minima and maxima. These parameters are stored in the computer memory together with data on character widths and line lengths ('measure'). Justification can then be achieved rapidly by subtracting from the measure the width of each

Table 2 Capital costs of a hot metal composing system, a conventional photosetting system and a practical ideal system

Hot metal £	Photosetting £	Practical ideal £
292,269	240,400	448,120

character as it is read, running back to the last space when the line overflows, and distributing through the inter-word spaces the total value of the space removed from the line end.

If the criteria for inter-word spacing do not allow this 'infilling' in a given case, then hyphenation is necessary. Computer hyphenation has been the *bête noire* of many photocomposing systems. The problem is that although it is possible to hyphenate by logic, there are, as in most grammatical rules, exceptions, and they are really too numerous to ignore. There was a short-lived move by some newspaper proprietors using photosetting to impose irregular hyphenation on their readers, but they discovered that the irritant factor was too great. Some systems enable the operator to decide hyphenation by displaying the word, but obviously delays of this nature are not ideal and the present practice is for computers to store an 'exception' dictionary, the routine being to search the dictionary for the word and use the recorded hyphenation break if it is there, or otherwise to hyphenate by logic.

With short-measure work such as newspaper setting, 50% of the total setting time¹ can be spent justifying by traditional methods, so keyboarding in an unjustified mode and leaving spacing and hyphenation activity to the computer has obvious attractions in terms of speed. The resulting possibility of boredom for the keyboard operator can, however, be a problem.

Once the attractions of computer justification were established, larger machines began to tackle fairly obvious tasks which needed more memory, considerable random access or more sophisticated programs: 'formatting' by the use of limited command key strokes to produce commonly used typographical combinations; tabular composition; scientific setting using a wide variety of characters; mathematical composition with superior and inferior characters, special symbols and variations from the base line of the type; the acceptance and re-formatting of wire service material, and so on.

Optical character recognition

Methods of input were scrutinised as the new technology threw into focus the inefficiency of 'double keyboarding'—once by the author or his typist and then by the typesetter. Optical character recognition (OCR) is one solution with which the newspaper industry in the USA, Britain and Europe is being wooed. Like most flirtations, this is running a bumpy course, but the principle is sound enough.

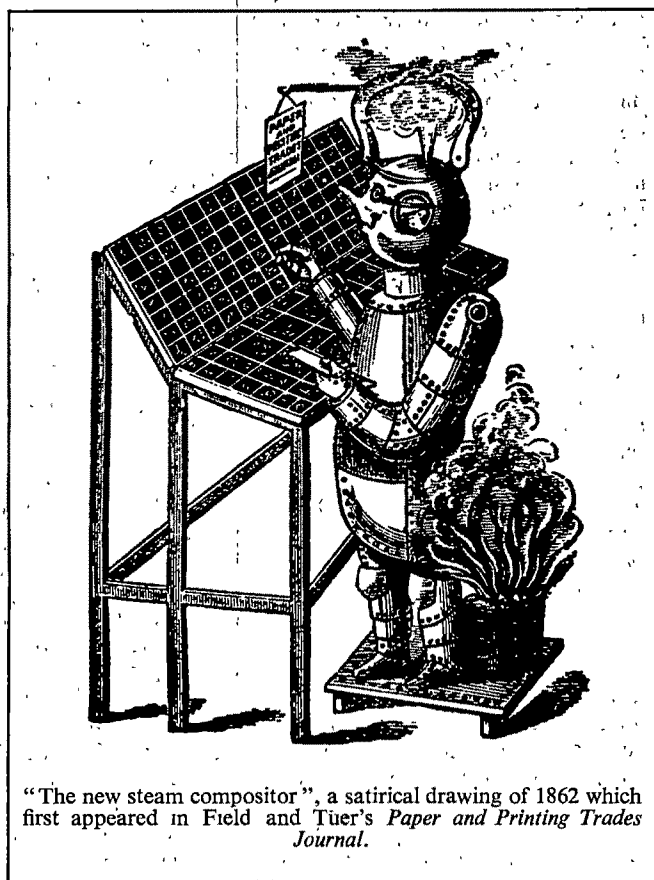
Typewritten manuscript is scanned by a 'reader' using photocells and the character which is recorded digitally is compared with master images held in binary form in the memory. Imperfectly formed characters will not be recognised on comparison with the memory data so fairly strict control of typescript quality is necessary. Specified typefaces must be used and carbon ribbons are used so that an even impression is made. Character recognition may still be thwarted by dirty typing heads, marks on the paper and so on, in which case the machine displays the offending line on a CRT with a query device such as a tick over the unrecognisable character.

Post-recognition logic may be very sophisticated with the acceptance of extraneous characters or character-combination codes for instructions such as 'delete previous word' or 'delete previous line'—given detailed code command programs and wide line spacing to allow for insertions, extensively edited copy can be accepted. Notes can be made on manuscripts with 'non-reading' pens, so most combinations of writing—editing—revising are possible. Output is normally to punched tape which can then be forwarded to the filmsetting computer.

Table 1 Running costs of a hot metal composing system, a conventional photosetting system and the practical ideal system

a Present running costs per year			
	Hot metal £	Photosetting £	Practical ideal £
Depreciation	15,048	29,580	68,040
Labour	209,962	183,130	85,179
Other costs	21,400	19,285	18,060
	246,410	231,995	171,279
b Forecast changes* in running cost indices due to rising labour costs			
	Hot metal	Photosetting	Practical ideal
Present	144	135	100
Two years	156	146	105
Five years	178	165	114

*Taking present 'Practical ideal' as 100 and assuming labour costs will rise 5% more per year than Depreciation and Other costs shown above, the changes in relative indices at two years and at five years due simply to this extra 5% in labour costs would be as shown.



Typewriter keyboards do not comprise a wide range of characters so the type of text which can be processed is restricted. Classified advertisement typesetting has been handled efficiently by OCR with the 'tele ad' girl who takes the advertisements by telephone producing the typescript which will become the typeset advertisement.

Extension of the use of OCR to general purpose setting is controversial, especially in newspaper work. If reporters can operate typewriters with the accuracy necessary for an OCR reader they can probably operate keyboards producing punched tape for the computer or sophisticated visual display terminals (VDTs) on-line to the computer.

Cost is an inhibiting factor, and so is the trade union viewpoint, but it is likely that for editorial work VDTs will be increasingly used in the editing process. Copy which has been sent to the computer whether by OCR, punched tape or other means, can be recalled to a CRT unit and edited—re-written if necessary—by keyboard and scroll devices, then restored. The implications for up-dating and 'editionising' on newspapers are significant.

An interesting sidelight on the output side is the increasing interest in computer output microfiche (COM). If typesetting has been digitised within a computer (or on magnetic tape for that matter) it can be projected on to a CRT and produced as microfilm or microfiche; in some industrial and academic applications the productions of 'hard copy' (paper output) can be eliminated and microfilm produced as the only form of origination. (The use of microfilm as computer input (CIM) is possible with a special OCR unit.)

Better hardware

The original typesetting minicomputers were small, simple units with no peripherals for storage. The tendency is still to use minicomputers but as additional applications have been spotted so the need for more random access has prompted the use of more and better hardware. Disk storage and magnetic tape are now widely used and the range of input and output devices has multiplied impressively.

Some of the new applications are closely linked with the typesetting function. When setting an index, for example, it saves considerable time to set from copy presented in random sequence and leave the alphabetical sorting to the computer. The index for *Nature* is handled in this way.

Other applications are less obvious. One of the most useful developments for newspaper houses has been the joint classified advertisement setting and accounting programmes. In a typical arrangement, classified advertisement information is input by OCR to create a master classified file which in the case of a daily newspaper is output daily for publication. The necessary data are included in each input to generate an invoicing and reporting cycle.

The master file is updated daily to carry forward advertisements which are booked for more than one insertion and delete those which have expired. The computer will sort into classifications and in some cases sort alphabetically within classifications. About 32 k of memory and reasonably extensive disk storage is needed to include this sort of operation.

In July this year a team from Portsmouth and Sunderland Newspapers in the UK completed a survey² into the 'practical ideal' for a provincial daily newspaper composing systems, based on equipment in use in the USA, the UK and Europe. Composing room staff would be "much reduced", said the report. For the size of newspaper considered, editorial copy would need 12 VDTs for input and a further 7 for sub-editing purposes. Copy which was written by reporters without direct access to the VDTs would be typed on machines with OCR heads and input by way of OCR readers. (See Fig. 1 for a schematic layout of a similar system.)

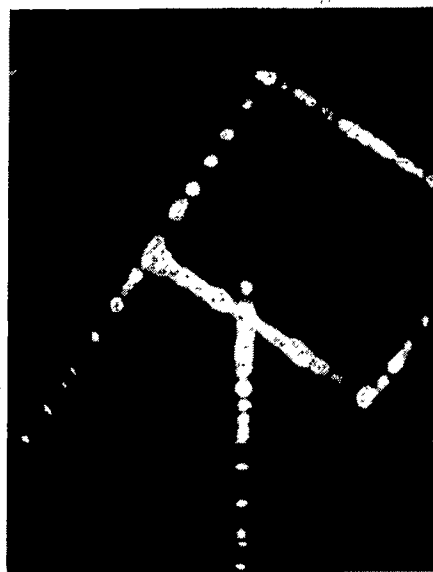
The advertisement department would need 11 VDTs (including one for the supervisor); non-VDT copy would be input by OCR. Some copy would need inputting without instructions, being recalled to the VDT for typographical display. Editorial and advertisement systems would be independent but each could back up the other.

Costs and the future

The costing aspect of a hot metal system, conventional photo-setting and the practical ideal is shown in Tables 1 and 2 (from ref. 2). When extended to show the cost of increased pagination, the practical ideal is highly advantageous.

In big newspaper houses and in some large commercial printers the practice is now to centralise typesetting, accounting,

Fig. 2 A magnified 'R' reproduced by lithography from a laser-etched litho plate developed by the London College of Printing research group.



payroll, circulation data and office uses in one large computer, but the outlay is prohibitive for the smaller company.

Complete page make-up within the computer and processing through to the platemaking stage without manual intervention is the ultimate aim of much print research. With simple formats, such as those in books, this is already in general use but with complicated newspaper pages there are problems.

Make-up of a page is possible on a large CRT but the input of graphics to a totally integrated page make-up system is still in its early stages of research. Theoretically the techniques are already available. Image data processing has entered the commercial market in various guises.

The Dicom Corporation of Minneapolis produces a series of 'digital image recorders' and 'image digitisers' which can store photographs in digital form and then print them back on to film. The picture is considered as a pattern of thousands of small patches of uniform brightness—'picture elements' or 'pixels'. It is scanned to sample the light intensity of each pixel, recognising a maximum of 256 intensity levels. This information is then digitised. To reprint the picture the information is recalled by focusing a CRT spot on to film, position by position, exposure time varying with the brightness needed.

The extension of this principle to the graphics area of full-page composition is obviously possible but a very high speed of input will be necessary and a vast amount of random storage will be needed for an operation of any size.

The general view seems to be that highly sophisticated full-page make-up is feasible but likely to be extremely expensive. At the London College of Printing (LCP), William J. Jones, the head of Pre-Printing Processes, and Dick Bowmaker, who runs the computer composition section, believe cheap hardware is the key to the next move in this area. The software, they say, is possible and the techniques available. Given the ever-accelerating development of inexpensive systems engineering, we are due for the next step. Expense is, as ever, the obstacle.

The LCP is intensely aware of the critical stage print technology has reached. Dr D. J. Morantz, the Deputy Principal, says the college is "endeavouring to secure facilities to research and teach the new areas of print and communications technology". Morantz himself leads a small research team at the college and has recently developed a technique for using a laser to etch litho plates.

Lasers have been used successfully to engrave plates for the gravure process, which prints from recessed images. For litho a subtler approach is necessary in view of the need to create a surface etching suitable for the rather difficult chemical requirements of the process.

The LCP team has used an argon ion laser on a specially coated 'plate' from which they have subsequently printed by lithography with promising results (see Fig. 2, for example).

They are now looking at the production of a direct coloured image by the same process and have succeeded in etching coloured images using specially formulated coatings. To project the idea, the production of a laser-etched plate direct from digitised data might be possible.

The new print technology is quite clearly a major headache for the print unions. In the USA there have been significant local breaks with print unions which could not come to terms with new techniques. Peter Watson, Digital Equipment Corporation's North European Technical Representative, estimates a move in the USA from 70% of newspapers unionised in the pre-computer days to 40% today, with the trend continuing.

In the UK, there have been minor breaks with the principal unions concerned and some serious disruption of production as new systems have come in. The National Graphical Association (NGA), the main union concerned, is inclined to take a fairly enlightened view at national level. "We've got to come to terms with the computer", says Roy Pearson, NGA National Organiser. "I think a careful programme of re-training is one of the answers. We accept that some compositors must become computer operators and even programmers."

But as Pearson admits, the problems in practice, at local level, are formidable. Computerisation of the national press composing rooms (which is now an economic necessity) must imply a massive fall-out of compositors, and potentially boring jobs for some of those who remain. None of the English national newspapers has yet reached an agreement on the full use of computer controlled composition. The human problems are certainly likely to be bigger than the technological ones.

This necessarily short review has deliberately ignored large areas of the printing scene. It has not mentioned colour processing or machine control, in which computers are used effectively. It has omitted details of developments such as the use of photopolymer plates which make photosetting methods suitable for the letterpress printing process.

The main purpose has been to sketch out the areas in which the skill of the 'printer' is being wholly or partly replaced by the computer, for this is the most significant aspect of the subject from a technological and human viewpoint.

I have therefore concentrated almost exclusively on the role of computers in composition (although it is likely anyway that the composition process will eventually extend through to the platemaking operation). This is the area in which the most dramatic modifications are taking place, the part of the industry which is changing from craft to computer.

¹ Holland, F. C., *The Role of Computers in Photocomposing* (PIRA Information and Training, Leatherhead, 1974).

² Miles, W. E., Wrightson, W. S., Penketh, D., and Lister, K., *Practical Ideal System of Copy Origination in Medium Sized British Provincial Newspaper* (Portsmouth and Sunderland Newspapers Ltd., Portsmouth, 1975).

Minicomputers are bursting out all over

Roger Woodham

The pre-eminent position of the central computer installation to which programs and data are submitted, to emerge hours or days later, is being challenged by the minicomputer, which offers considerable computer power to individuals at the time and place they require it.

ASKING for a handy definition of a minicomputer is rather like asking the length of a piece of string: few people are prepared to commit themselves except in the vaguest of terms.

Yet minicomputers have completely changed the face of computing by making available to individuals—be they scientists, medical personnel, civil engineers or teachers—the kind of personal, 'hands-on' computing facilities that the large mainframe computer, in its cloistered and pampered surroundings, can never provide.

When pressed, however, people in the computer field will generally classify a particular computer as a 'mini' on the basis of its price. Broadly speaking, a minicomputer system costs between £1,500 and £50,000, at which price it turns imperceptibly into a 'midi', costing anywhere between £50,000

Table 1 Principal minicomputer manufacturers

Company	Market share 1974 (%)	Minicomputer revenues (\$ million)
Digital Equipment	35	410
Hewlett-Packard*	13	150
IBM*	12	145
Data General	8	92
General Automation	6	65
Honeywell	4	50
Varian Associates	3	34
Interdata	3	30
Modular Computer	2	26
Computer Automation	2	22
Systems Engineering Labs	2	18
Microdata	1	15
Digital Computer	1	10

*Includes internal installations.

and £150,000; the midis, in turn, can be divided off from the 'maxis' or 'majors', which cost more than £150,000. At the other end of the scale there is the microcomputer, the 'computer on a chip', which may cost as little as a few hundred pounds but which is so much more than a glorified hand calculator.

Growth industry

Most people would associate the name DEC (Digital Equipment Corporation) with minicomputers, and certainly that company makes the lion's share of all minicomputer sales (see Table 1). But then it was a trend setter as long ago as 1958 and has been selling its PDP series, among others, ever since. Data General, on the other hand, is the prime example of a blossoming newcomer, founded only in 1968 by a former employee of DEC who put his own ideas on the development of minicomputers into practice on his own account when DEC failed to adopt them. Since then Data General has gone from strength to strength, doubling its annual sales (which have totalled 20,000 machines in its seven years of life) every year.

During the past eight years or so, the costs of minicomputers have dropped considerably, as the price of electronic components to carry out a given function have decreased. In 1967, for example, the basic PDP8, perhaps the most widely used of DEC minicomputers, cost more than £7,000; nowadays the price of the PDP8A is little more than £1,000.

It would be a mistake, however, to think that most minicomputers are sold to individuals to use in their laboratories or offices. DEC estimates that 50–60% of its sales (by value) are not made to the final user but to another company which incorporates the computer into its own equipment, for example a mass spectrometer, where it will be used to control the operation of the equipment and process the data that emerges.

It would also be a mistake to imagine the minicomputer as simply a box that fits on to a table top and is accompanied by a typewriter input and output. That may have been the case in the late 1960s, but now a minicomputer is much more of a system, with the minicomputer proper accounting for relatively little of the cost compared with line printers, visual display units, graphics terminals, disk stores and the like.

In many ways the whole philosophy surrounding the manufacture of minicomputers distinguishes them from the larger machines. Not only do companies like Data General and DEC operate with far fewer of the trappings like prestige offices than many of the companies manufacturing mainframe computers, but the very nature of the minicomputer means that vast armies of engineers and service personnel are not required. One of the main attractions of minicomputers is

that they can be operated in the relatively dusty, humid and warm conditions of a normal laboratory or office, safe in the knowledge that they will not break down with the monotonous regularity of some mainframe machines. It is true that the criticism has been levelled against some companies manufacturing minicomputers that their after-sales service is not good, but on the whole minis seem to provide, pound for pound, much better value for money than the larger machines. One can understand why the manufacturers of big machines sometimes refer to minicomputing as 'underground computing'. It certainly gives them something to think about.

Minicomputers and scientists

Because minicomputers are tailored by their users to fit precisely the task required of them, it is difficult to generalise about the ways in which scientists use them. Almost any laboratory that requires data logging, control of equipment in real time or arithmetical calculations of any kind can profitably make use of a mini. If that mini can also be connected in to a large mainframe computer as a 'front-end machine', then the tasks

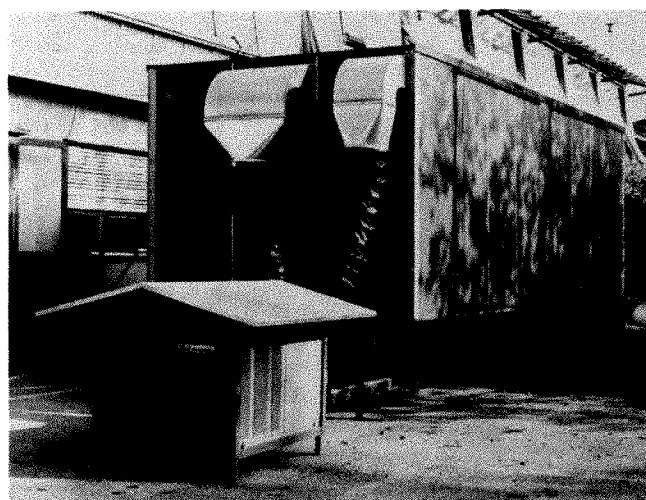


Fig. 1 This travelling computer laboratory developed at CERN contains three minicomputers, and can easily be moved from place to place.

which can be carried out (particularly 'number crunching') can be that much more sophisticated and complex.

A crystallographer, for example, might use a minicomputer system to provide views of a complex molecular structure from different angles whereas, an astronomer might incorporate a minicomputer into his telescope control equipment so that a given part of the sky can be tracked for long periods of time. Engineers, on the other hand, might well set up a mathematical model for a bridge—or an aircraft wing—and use the minicomputer to display on a visual display unit or graph plotter the consequences of certain input data (loading, sizes of beams, or whatever). Some people have found minicomputers a sufficiently attractive proposition to group them together into a travelling laboratory (Fig. 1).

The examples are so numerous that I shall describe in more detail the use made of minicomputers in a particular university department, the Department of Aeronautics at Imperial College, London. Typical is not the word to use, as no minicomputer system is typical, but certainly some of the characteristics are similar to some of those of other installations.

The department's minicomputing facilities certainly take up

rather more than a table top, a medium sized room in fact. They include the usual peripherals and in addition magnetic tape decks for data storage and the necessary equipment to link the department's facilities to the main college computer, a CDC machine. It is thus possible to collect, store and do calculations on data both locally and in the CDC machine as the need dictates.

The Aeronautics Department operates two hypersonic wind tunnels—one a 'gun tunnel' in which everything is over in 10 ms or so (but can be repeated about every 20 min) and the other a continuous low density nitrogen wind tunnel which requires accurate knowledge of flow rates, pressures and so on to establish the conditions of operation. Before the mini-computing facilities were acquired a year or so ago, the pressure profiles for the gun tunnel were recorded photographically using eight oscilloscopes and much of the subsequent data extraction was done manually. Now, however, the profiles are recorded and analysed by the minicomputer system, with the minimum of human intervention.

HELLO. THIS IS AN EXPERIMENT IN TAKING DETAILS OF MEDICAL SYMPTOMS BY A COMPUTER. IT IS DESIGNED TO HELP DOCTORS IN THEIR WORK, AND TO IMPROVE MEDICAL FACILITIES FOR THE PATIENTS AS WELL.

WE WOULD LIKE YOU TO HELP US WITH THIS AND IF YOU ARE PREPARED TO WOULD YOU PUSH THE BUTTON MARKED 'YES'. IF NOT PRESS THE BUTTON MARKED 'NO', AND IF YOU DON'T UNDERSTAND PRESS THE '?' BUTTON.

GO AHEAD AND PUSH ONE OF THE BUTTONS NOW? 0

GOOD, THANKS VERY MUCH. EVERYTHING THAT YOU SAY WILL BE TREATED IN THE STRICTEST CONFIDENCE AND ONLY DOCTORS AND NURSES WILL MAKE USE OF THE INFORMATION THAT YOU HAVE GIVEN. ARE YOU QUITE HAPPY ABOUT THIS? PUSH 'YES' IF YOU ARE, 'NO' IF YOU'RE NOT AND '?' IF YOU DON'T UNDERSTAND? 0

GOOD. THIS IS A COMPUTER TALKING TO YOU. I CAN ASK YOU QUESTIONS BUT I CAN ONLY UNDERSTAND SIMPLE ANSWERS, AND YOU GIVE ME THESE ANSWERS WHEN YOU PUSH ONE OF THE THREE BUTTONS IN FRONT OF YOU. THEREFORE ALL THE QUESTIONS CAN BE ANSWERED IN THIS WAY. DO YOU UNDERSTAND EVERYTHING I'VE SAID UP TO NOW? 0

BY THE WAY, IN YOUR JOB DO YOU EVER WORK AT NIGHTS? PRESS ONE OF THE BUTTONS NOW? 1

OK. NOW I WANT TO ASK YOU SOMETHING ABOUT YOUR CHEST. BEFORE YOU PUSH ONE OF THE BUTTONS I'D JUST LIKE TO REMIND YOU THAT IF YOU DON'T QUITE UNDERSTAND WHAT I MEAN BY THE QUESTIONS, THEN PRESS THE '?' BUTTON. TELL ME, DO YOU USUALLY COUGH FIRST THING IN THE MORNING IN THE WINTER? PUSH ONE OF THE BUTTONS NOW? 0

DO YOU USUALLY COUGH DURING THE DAY IN WINTER? PUSH ONE OF THE BUTTONS? 0

DO YOU COUGH LIKE THIS ON MOST DAYS FOR AS MUCH AS THREE MONTHS EACH YEAR? 6

OBVIOUSLY I CAN'T BE TOO PRECISE ABOUT THIS, BUT WOULD YOU SAY THAT YOU COUGH LIKE THIS ON MOST DAYS FOR AS MUCH AS THREE MONTHS IN A YEAR. PRESS YES OR NO THIS TIME? 1

WOULD YOU SAY THAT WHEN YOU GET A COLD IT GOES DOWN TO YOUR CHEST? 0

WHEN YOU GET A COLD, HOW LONG DOES IT TAKE TO CLEAR UP. FIRST, WOULD YOU SAY IT ONLY TOOK THREE OR FOUR DAYS? 1

IS IT ABOUT A WEEK? 0

Fig. 2 A conversation between a patient and a computer (from ref. 1). Replies: 1, no; 6, ?; 0, yes.

In the case of the continuous wind tunnel, it used to take up to two days to find the operating conditions by submitting data in the conventional way to the central college computer. Now they have the answer in 30 s.

As well as using the mini system for developing and testing mathematical models, for example of the behaviour of a helicopter rotor blade in various conditions, the department will also use it to control a milling machine and to develop electronic circuits. In the latter case, the computer will simulate the operation of each component that goes to make up a

circuit and thus will be used to predict the performance of the completed circuit.

Medical applications

Minicomputers are also becoming of increasing importance in medicine—both in the diagnostic field, where they are slowly but surely reshaping the traditional doctor-patient relationship, and in the laboratory, where they are increasingly relieving medical technicians of time-consuming chores. For example, colorimetric tests carried out to detect the presence of given substances in body fluids using sequential multi-channel analysers can be speeded up and made more accurate by using a minicomputer. Not only can the data be produced in a form suitable for use on the wards—a transcription process previously left to the technicians—but the analysers can be automatically recalibrated against a standard solution every so often, and an automatic correction made to all measurements as the instrument slowly and inevitably drifts away from its calibrated state. The same goes for many other instruments used to make pathological measurements.

In the case of medical interviewing by computer, the aim is to get a patient to respond to a sequence of questions about his state of health which either gives the doctor sufficient information to make a diagnosis or establishes that the patient is for some reason unable to communicate with the computer (see, for example, Fig. 1). The keeping of medical records is a whole new area again in which minicomputers seem set to spark off a revolution. It is true that hospitals have tried keeping records on large central computers before, but some schemes have come unstuck because of a reluctance on the part of doctors to write up reports in long-hand and then, perhaps the next day, rewrite them into the computer. They rightly think that is a waste of time, but could undoubtedly be persuaded to use a system which was fully accessible to them the first and only time they write up their reports and observations.

Industry and energy

The capability of the minicomputer for process control clearly has many implications for manufacturing industry, particularly as the operation of manufacturing plant in sub-optimum conditions often means that energy is being wasted. The aluminium industry is just one that has benefited from minicomputer control, in that several companies use minis to ensure that the resistance of the electrolytic cells in which the alumina is reduced does not vary, and with it the current consumption. No foreman can hope to check the voltage drop across each cell and adjust the electrode positioning sufficiently quickly that the cells operate in non-optimum conditions for less than a few minutes, and the outcome, when multiplied up, is a larger consumption of energy than necessary. Plainly any industrial process which uses any kind of control loop can profitably be linked up to a 'dedicated' minicomputer, be it steel rolling or the production of chemicals.

Decreasing size

As microprocessors come into their own, the price of minicomputers is likely to continue to fall, with the bulk of the space inside a minicomputer being taken up with connections to and from a single chip. Microprocessors are, however, likely to find their greatest application as preprogrammed computers within other pieces of apparatus. Whatever else happens, underground computing is here to stay.

¹ Evans, C. R., Kinchin, C. G. J., Price, H. C., and Whittle, P. B., *NPL Report Com 73* (National Physical Laboratory, Teddington, 1974).

articles

Diversity of cross-bridge configurations in invertebrate muscles

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X-ray diffraction patterns from relaxed invertebrate muscles reveal the thick filament symmetries and cross-bridge configurations. The cross bridges are substantially angled to the filament axes. The results on symmetry are generally consistent with Squire's model.

X-RAY diffraction studies of striated vertebrate and insect muscles^{1,2} have not yet established the symmetry of the array of myosin cross bridges on the thick filaments, nor the configurations of the resting cross bridges. Squire^{3,4} has proposed a scheme for thick filament structure that predicts a relationship between the rotational symmetry and the diameter of the filament backbone, and on this basis has suggested possible values for the rotational symmetries of vertebrate striated and insect muscle thick filaments. These proposals are supported by recent chemical evidence^{5,6}.

We describe here the detailed low angle X-ray diffraction patterns given by relaxed striated muscles from three invertebrate animals: *Limulus* (horseshoe crab), *Homarus* (lobster) and *Placopecten* (scallop). These patterns differ from those given by vertebrate and insect muscles in one important respect; the diffraction from myosin shows sampling from the hexagonal filament lattice only on the equator. In two cases, this enables us to determine the rotational symmetries from the reflection profiles; moreover, the relative intensities of the layer lines can be used in a straightforward way to determine the configurations of the resting cross bridges. The cross bridges seem to be markedly tilted in the resting state, and to take up diverse configurations and positions. Our conclusions on thick filament symmetry are broadly consistent with Squire's model⁴.

Description of diffraction patterns

We describe here only those features of the diffraction patterns that relate to thick filament symmetry and cross-bridge configuration. Other aspects of the patterns, and full details of their analysis, will be described elsewhere.

The pattern from *Limulus* muscle has been described by us previously⁷ (Fig. 1a); it is characterised by a series of strong myosin layer lines indexing on a repeat of $3 \times 146 = 438$ Å. The first and third are the strongest of these; the fourth is of moderate intensity; and the second, fifth and sixth layer lines are weak. The meridional reflection on the 146-Å (third order) layer line has a clear subsidiary maximum associated with it, 270 Å from the meridian.

In the pattern from *Homarus* muscle (Fig. 1b) the only layer lines which can be unambiguously assigned to myosin

are those at 146 and 73 Å. These have strong meridional peaks, and the 146-Å layer line has a clear subsidiary peak 230 Å from the meridian.

The *Placopecten* muscle gives the most detailed pattern of the three (Fig. 1c); the myosin layer lines are clear and index on a repeat of $10 \times 146 = 1,460$ Å, the first strong non-meridional layer line being at 487 Å (third order), with weaker ones at 208 Å (seventh order) and 112 Å (13th order). The 146 and 73-Å layer lines are remarkably detailed; at least two subsidiary maxima accompany the meridional peak in each case, falling at different positions on the two layer lines (270 and 145 Å off meridian for the 146-Å layer line and 365 and 165 Å for the 73-Å layer line). Additional meridional reflections occur at 98 and 58 Å, which index as the third and fifth orders of $2 \times 146 = 292$ Å; these have a different profile, with no apparent subsidiary maxima.

Model calculations

We have compared the observed diffraction patterns with those calculated for model thick filaments with different helical symmetries and cross-bridge configurations. We assumed that each bridge corresponds to a single myosin molecule, and that the diffraction from the tails of the molecules (forming the filament backbone) could be neglected. A cross bridge was modelled as either a single cylinder (150 Å long \times 56 Å diameter), or as two cylinders (each 150 Å long \times 40 Å diameter) representing the two subfragment-1 (S1) subunits.

Figure 2 illustrates some relevant concepts of helical symmetry. Sections of a single type of surface lattice may be rolled up to form different multi-stranded helices (Fig. 2a); these differ in rotational symmetry (order N) and diameter, but have a very similar local geometrical relationship between neighbouring lattice points. This relationship can be determined easily from the layer line spacings in the diffraction patterns, which give directly the angular relation of one layer of cross bridges to the next (compare definition of screw order in Fig. 2). The axial translation between layers of cross bridges is within 1 or 2% of 145 Å in all thick filaments. The crucial point is that the different helices give very similar diffraction patterns, in which the different diameters are manifest only in the detailed profiles of the reflections. Qualitative aspects of the intensity distribution among the layer lines can be understood by reference to the (n,l) -plot; alignment of cross-bridge density with a particular set of helical tracks produces enhancement of the corresponding layer lines, irrespective of the rotational symmetry (Fig. 2b and c).

The following points were established by our calculations of the cylindrically averaged intensity distribution⁸ expected from helical arrays of cross bridges.

(1) The 146-Å layer line has a strong meridional peak, with a weaker subsidiary peak. For models with cross-bridge lengths

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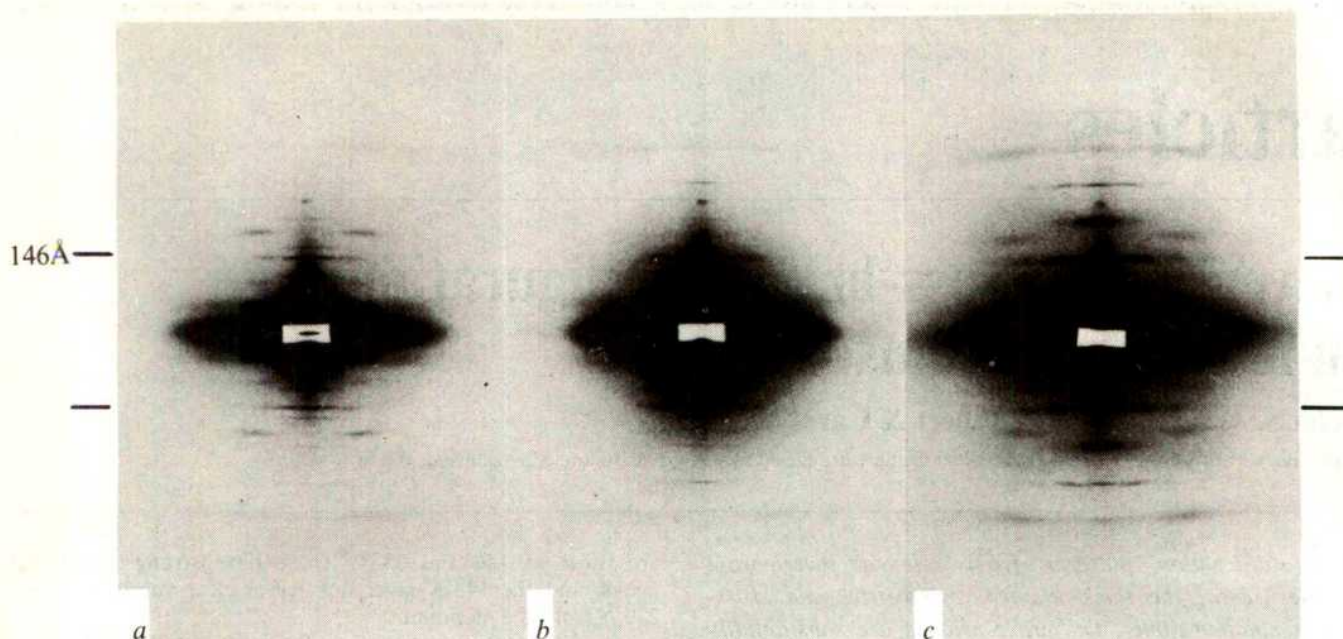


Fig. 1 Low angle X-ray diffraction patterns obtained from strips of muscle about 1 mm in diameter, mounted in a cell under tension at 4 °C. Glycerinated strips (prepared as before⁷) were immersed in a relaxing buffer containing ATP; living muscles were kept in an appropriate aerated Ringer solution. A mirror-monochromator X-ray camera with a specimen-to-film distance of 400 mm was used. The X-ray source was an Elliott GX6 rotating anode loaded at 600 W. Multiple packs of Ilford Industrial G or Kodak Industrex AA film were used to record the patterns; intensity data were obtained from them by scanning on a Joyce-Loebl microdensitometer or an Optronics rotating drum scanner. Background was subtracted using scans on either side of the layer line in question; for more detailed analysis of the profiles of meridional reflections and their subsidiary maxima, Optronics scans on 25 μ m or 100 μ m rasters allowed a similar subtraction of background to be made at each point along the layer line. Reflection profiles were further corrected by deconvoluting the profile of the main beam, assumed to be Gaussian. *a*, Pattern from abdominal muscle of *L. polyphemus* (see ref. 7). Glycerinated muscle in relaxing buffer (40 mM KCl, 8 mM MgCl₂, 1 mM EGTA, 5 mM NaATP, 10 mM histidine, 0.1 mM sulphadiazine, pH 7.0). *b*, Pattern from deep abdominal extensor muscle of *H. americanus* (see ref. 11). Glycerinated muscle in relaxing buffer. *c*, Pattern from striated (translucent) adductor muscle of *P. magellanicus*; living relaxed muscle in *Limulus* Ringer solution⁷.

up to about 200 Å, the distance of this subsidiary from the meridian depends on the radius at which the cross bridges are centred, and is only slightly affected by bridge configuration. Axial tilt (see Fig. 2) of the cross bridges weakens the meridional peak more than the subsidiary peak, so that the ratio of intensities of the two reflects the amount of tilt. The intervening minimum becomes less distinct when tilt is increased without azimuthal twisting.

(2) On the other (non-meridional) layer lines the radial positions of the maxima depend not only on bridge radius but also on rotational symmetry of the filament. The axial spacings of these layer lines are determined by the helical symmetry; the strongest layer line is that corresponding to the pitch of the myosin helix (*P* in Fig. 2*a*), and for a given symmetry and bridge radius its intensity and profile are remarkably insensitive to tilting and twisting of the cross bridges. Other layer lines are more sensitive to changes in bridge configuration, as expected from the (*n*,*l*)-plot (Fig. 2*b*).

(3) When the S1 subunits in each bridge are separated, additional effects are observed; the subsidiary maxima on the 146 and 73-Å layer lines are shifted in position, and the intensities of all the layer lines are further modulated by interference between the two subunits.

Interpretation of diffraction patterns

The spacings of the layer lines in the horseshoe crab pattern indicate that there are three layers of cross bridges separated by 146 Å in the repeat length of 438 Å (compare Fig. 2*a*). The profile of the 146-Å layer line shows that the cross bridges are centred at a radius of about 165 Å from the helix axis. The profiles of the other non-meridional layer lines are accounted for if there are three or four cross bridges at each level in the thick filament (compare Fig. 2*a*). The intensity of the 146-Å meridional is no greater than that of the first layer line at 438 Å, suggesting that the cross bridges are tilted axially, by at least 30° from the normal to the filament axis. The striking

modulation of the layer line intensities can be explained by a substantial degree of azimuthal twist of the cross bridges; combinations such as 60° twist and 30° tilt give a good fit for the profiles and intensities of layer lines 1–6 (Fig. 3*a*). With our present data, there is no need to invoke the separation of S1 subunits.

The *Homarus* pattern does not enable us to determine the symmetry of the surface lattice, but we find from the profile of the 146-Å layer line that the radius of the cross bridges from the axis is about 140 Å. The unusually small (3:1) ratio of meridional to off-meridional intensity on the 146-Å layer line indicates some axial tilt of the cross bridges. The closeness of the bridges to the axis suggests a reason for the absence of non-meridional layer lines: models with such a small radius and a high (for example, sixfold) rotational symmetry give very weak layer lines if the bridges are even slightly twisted azimuthally (compare Fig. 3*b*).

The spacings of the layer lines in the *Placopecten* pattern indicate that layers of cross bridges separated by 146 Å are related by a slightly different screw operation from that in *Limulus*: the ratio of the spacings of the strong 487 and 146-Å layer lines is here 10/3, so that the repeat length is $10 \times 146 \text{ Å} = 1,460 \text{ Å}$, although the angular relationship between 146-Å layers is only slightly different from that in *Limulus*. The screw order is defined in Fig. 2. The layer line profiles are more complex in this pattern than in the other two, but we find that the striking difference in profile between the 146 and 73-Å layer lines is well explained by certain specific bridge configurations. The bridges must have large axial extent when projected on to the axis, but the presence of clear minima in the profiles shows that this does not result from tilting single rods: instead, a good fit results when the two S1 subunits are separated axially, for example, by tilting about 20° up and 20° down, respectively. The profiles, interpreted in this way, indicate that the bridges are centred about 170 Å from the filament axis; the non-meridional layer lines can then be

fitted when the rotational symmetry is six- or sevenfold. Since the non-meridional layer lines are strong, azimuthal twisting of the bridges must be small (compare Fig. 3c). The presence of meridional reflections at odd orders of 292 Å in patterns from relaxed (but not rigor) muscles can best be explained by small axial perturbations of the bridges at alternate 146-Å levels along the filament (Millman and Bennett, unpublished).

In summary, the cross bridges in these muscles seem to lie at similar radii from the filament axes in spite of differences

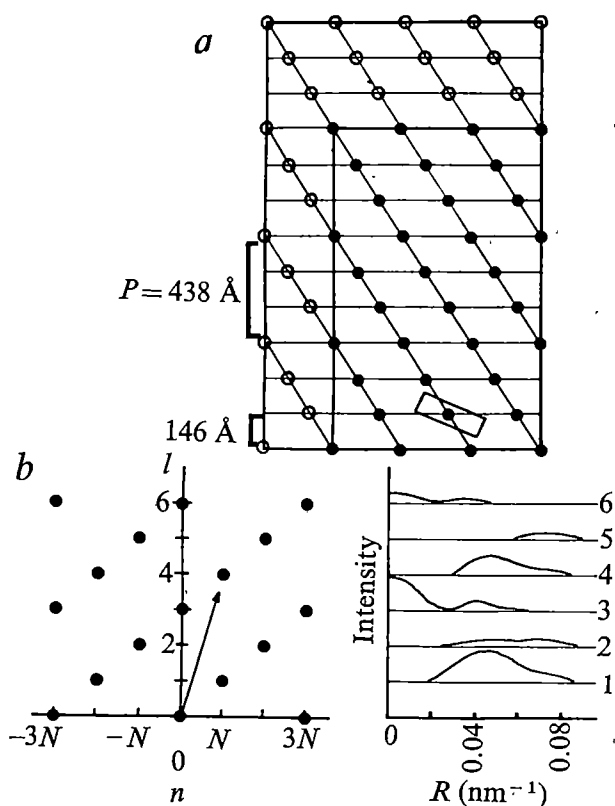


Fig. 2 *a*, Radial projections of two multi-stranded helices derived from the same surface lattice, but with differing rotational symmetries ($N = 3, 4$). The blocked area represents schematically the appearance in radial projection of a cross bridge tilted axially and azimuthally. We define axial tilt as the angle between the long axis of the bridge and the plane normal to the filament axis, and azimuthal twist as the angle (measured in this plane) between the bridge axis and the radius from the helix axis to the bridge's centre of mass. The two helices have symmetries S_3C_3 and S_3C_4 respectively, according to a notation suggested to us by D. L. D. Caspar: the notation $S_\sigma C_N$ indicates combination of a screw axis S_σ (of screw order $\sigma = 2\pi/N\phi$ where ϕ is the azimuthal rotation between units at different levels) and a point group C_N (where N is the order of rotational symmetry). Note that σ is the number of units per turn of the helix divided by the rotational symmetry; thus helices with the same value of σ but different values of N have the same local geometry in the surface lattice. Moreover, the diffraction pattern from a helix gives the value of σ directly. Thick filaments of *Limulus* have either S_3C_3 or S_3C_4 symmetry (see text); vertebrate striated muscle filaments would be S_3C_2 (Huxley and Brown's 6₂-helix model¹), or S_3C_3 or S_3C_4 (Squire's models^{3,4}). In *Placopecten* muscle, the layer line spacings in the diffraction pattern show that $\sigma = 10/3$ (see text); and the rotational symmetry is either six- or sevenfold: thus the filament has $S_3C_3C_6$ or $S_3C_3C_7$ symmetry on this notation. *b*, (n, l) -plot for the surface lattice in *a*, which describes the diffraction from any of the helices derived from it. The arrow indicates the direction in the diffraction pattern of the spike of intensity corresponding to the cross bridge orientation shown in *a*. Changes in orientation of this spike cause larger changes of intensity on layer lines far from the origin than on those closer in. *c*, Cylindrically averaged intensities calculated for the helix with $N = 3$, with single-rod cross bridges tilted 30° axially and 60° azimuthally (as in *a*). The weak second layer line, the strong fourth layer line, and the outward displacement of the peak on the fifth layer line are as expected from consideration of the (n, l) -plot.

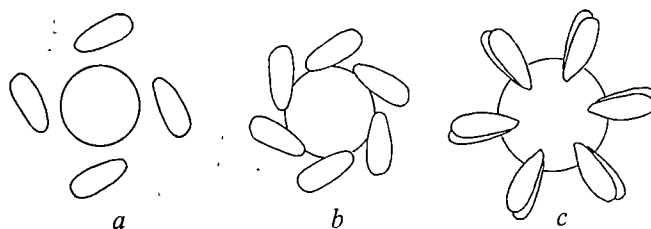


Fig. 3 Scheme illustrating the diversity of bridge location and configuration in myosin filaments: *a*, *Limulus*; *b*, *Homarus*; *c*, *Placopecten*. The bridges at one level in each filament are shown, and the circle represents the backbone diameter as estimated from electron microscopy (see text references). Rotational symmetries for *Limulus* and *Placopecten* are those which give agreement with present data; the symmetry for *Homarus* is hypothetical (see text).

in screw and rotational symmetries. The orientations of the cross bridges vary: *Limulus* bridges, viewed as single rods, are tilted axially and twisted azimuthally; in *Homarus* they are also tilted axially, and may be slightly twisted azimuthally; and in *Placopecten* muscle there is evidence for axial splaying of the S1 subunits, with little or no azimuthal twist, and a periodic perturbation of the bridges at alternate levels.

Implications of the patterns

These are the first X-ray diffraction patterns that reveal the rotational symmetries of the thick filaments: three- or fourfold in *Limulus* muscles and six- or sevenfold in *Placopecten* muscles. Squire's model predicts a relationship between the rotational symmetry and the diameter of the filament backbone, but we have not been able to determine this diameter directly from the X-ray patterns because of limitations of the equatorial data. Electron microscope studies suggest that thick filaments of *Limulus* are about 195 Å in diameter¹⁰ whereas filaments of *Homarus* and *Placopecten* may be larger (210 and 225 Å, respectively^{11,12}). Until we obtain more precise values for the backbone diameters in these muscles, we cannot test Squire's model rigorously; but our results on symmetry, together with the values for diameters obtained by electron microscopy, seem to be consistent with his proposal on the structure of thick filament backbones.

Our results emphasise that although there seems to be a close relationship between the backbone structures in the thick filaments of different muscles, the cross bridges adopt various configurations. One similarity, however, is that in all these muscles the relaxed cross bridges are tilted away from the normal to the filament axis. If the bridges are attached to actin in a right-angled configuration before the power stroke of the contractile cycle, then some angular movement of the bridges must occur on activation or initial attachment in these muscles.

Our results on cross-bridge configuration depend on assumptions about the dimensions of the cross bridges. We have used those dimensions found for vertebrate S1 attached to actin by three-dimensional reconstruction from electron microscope images¹³. The bridges in our three relaxed muscles may depart from these dimensions, but must be elongated: we found no way of explaining the diversity of patterns other than in terms of specific orientations of rod-shaped bridges. As more precise information becomes available, our solutions will have to be revised accordingly, but we believe that the models (Fig. 3) illustrate the variety of bridge configurations required to account for the X-ray observations.

The diffraction patterns from relaxed vertebrate and insect muscles^{1,2} contain, in principle, similar information on filament symmetry and cross-bridge configuration, but the presence of sampling necessitates the use of correction factors for the meridional intensities and prevents observation of the full layer-line profiles. Sampling in the pattern from relaxed frog muscle is reduced when the muscle is stretched to a length where thick and thin filaments no longer overlap¹⁴; the fourth

layer line is then observed to be prominent, as in the pattern from relaxed *Limulus* muscle, suggesting that similar tilting and twisting of the bridges occur in this muscle. Further evidence for tilting is provided by the absence of a clear minimum on the 143-Å layer line. Squire¹⁵ reports that analysis of the sampled pattern leads to a similar conclusion.

The three invertebrate muscles we have studied possess three different regulatory mechanisms (actin-linked, myosin-linked, and double regulation¹⁶). We are now using X-ray diffraction to determine how these regulatory systems influence attachment of the cross bridges to actin.

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³¹P NMR studies of NADPH and NADP⁺ binding to *L. casei* dihydrofolate reductase

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The ³¹P NMR spectra of NADPH and NADP⁺ in their complexes with dihydrofolate reductase show effects from three-bond ¹H-³¹P coupling constants which indicate that the conformation about one of the C₅'-O₅' bonds changes when the coenzyme is bound to the enzyme. ³¹P chemical shifts demonstrate that the 2'-phosphate group is in the dianionic state in the complex.

DIHYDROFOLATE reductase catalyses the NADPH-linked reduction of dihydrofolate to tetrahydrofolate. As part of a wider study of the binding of ligands to the dihydrofolate reductase from *Lactobacillus casei*, we have used high resolution nuclear magnetic resonance (NMR) spectroscopy to study the complexes of NADPH and NADP⁺ with the enzyme^{1,2}. These experiments showed that both coenzymes bind tightly to the enzyme, with a stoichiometry of 1 mol per mol enzyme, and provided some evidence that a change in the conformation of the enzyme accompanies coenzyme binding.

In ¹H NMR studies of the complexes of tightly bound ligands, it is often difficult to distinguish the resonances of the bound ligand from the complicated spectrum of the protein. This problem can be circumvented by selective deuteration, or by studying nuclei other than protons. We have used ¹³C NMR to study the binding to dihydrofolate reductase of NADP⁺ enriched to 90% with ¹³C at the carboxamido carbon on the nicotinamide ring². A logical extension of this kind of experiment is to monitor the ³¹P resonance signals of the coenzyme. ³¹P is the naturally occurring isotope of phosphorus, and is relatively sensitive to NMR detection. Several other workers have used ³¹P NMR to study ligand binding to macromolecules^{3–9}, and Hoult *et al.* used this technique to study phosphorylated metabolites in muscle¹⁰. We have begun our ³¹P NMR studies of coenzyme binding to dihydrofolate reductase by examining the binary complexes of the enzyme with NADPH and NADP⁺, and the results of these experiments are reported here.

³¹P chemical shift measurements

The ¹H noise-decoupled ³¹P NMR spectrum of NADPH

(Fig. 1a) consists of two absorption bands in the intensity ratio 1:2. The low field signal (0.47 p.p.m.) arises from the 2'-phosphate group, and that at -13.78 p.p.m. arises from the two phosphorus nuclei in the pyrophosphate group, which accidentally have the same chemical shift¹¹. In the binary complex of NADPH with dihydrofolate reductase (Fig. 1b) a markedly different spectrum is observed. The 2'-phosphate resonance is shifted downfield by 2.19 p.p.m., whereas the resonances of the two pyrophosphate phosphorus nuclei are both shifted upfield, but to very different extents (0.16 and 2.65 p.p.m.). Since these latter two phosphorus nuclei are now magnetically non-equivalent, their signals appear as an AB quartet, with ²J_{P-O-P} = 20.8 Hz. The small intensity difference between the two halves of the quartet is almost certainly due to a small difference in relaxation time. At present, it is not possible to assign the two pyrophosphate ³¹P NMR signals in the complex to the two individual phosphorus nuclei. Further addition of NADPH to give a molar ratio of NADPH to enzyme of 2:1 led to the appearance of additional resonances at the positions of those of free NADPH, with no other changes in the spectrum (not shown). Clearly there is only slow exchange (on the NMR time scale) between free and bound NADPH.

Similar experiments were carried out with NADP⁺. In the binary complex of NADP⁺ and dihydrofolate reductase, the chemical shift of the 2'-phosphate resonance is identical to that found for the NADPH complex (though the ³¹P chemical shifts of this group are different in free NADP⁺ and NADPH, see below). Again, both pyrophosphate signals are shifted upfield to different extents (there is a small non-equivalence of the two pyrophosphate phosphorus nuclei in free NADP⁺; see also ref. 11). The magnitude of the larger of the two shifts is, however, distinctly less than that seen with NADPH. The chemical shifts are summarised in Table 1. The ³¹P signals from the enzyme-NADP⁺ complex are somewhat broader than those from the enzyme-NADPH complex. This is probably due to an exchange contribution to the linewidth in the former complex, as observed in earlier ¹³C studies².

In the free coenzymes, the 2'-phosphate group has a pK of 6.1 for NADPH and 6.4 for NADP⁺. The ³¹P resonance from this group shows a corresponding change in chemical shift with pH (see also ref. 11), the total titration shift being 3.9 p.p.m. (Fig. 2). In contrast, in the complexes with the enzyme the

2'-phosphate resonance of both NADPH and NADP⁺ was independent of pH within the range pH 4.5 to 7.5, occurring at a position about 1.7 p.p.m. downfield of the position of the dianionic form of the 2'-phosphate in the free coenzymes. The chemical shifts and $^2J_{P-O-P}$ coupling constant of the pyrophosphate group were also independent of pH over the same range.

³¹P-¹H spin-spin coupling constants

The remarkably narrow resonance lines ($\Delta\nu_{1/2} \sim 7$ Hz) observed in the ¹H noise-decoupled ³¹P spectra of the NADPH-enzyme complex encouraged us to examine the single-resonance ³¹P spectrum. In the single-resonance ³¹P spectrum of free NADPH

Table 1 ³¹P chemical shifts of NADPH and NADP⁺ and their complexes with dihydrofolate reductase

	2'-Phosphate	Pyrophosphate	
		A	B
NADPH (pH 6.9)	0.47		-13.78
NADPH-enzyme	2.66	-13.94	-16.47
NADP ⁺ (pH 6.9)	-0.22		(-14.47)
			(-14.15)
NADP ⁺ -enzyme	2.72	-14.32	-16.23

The chemical shifts are in p.p.m. and were externally referenced to 50 mM K₂HPO₄ solution (pH 8.0) contained in a capillary. Positive shifts to low field.

(Fig. 1d) the 2'-phosphate resonance shows a doublet splitting ($^3J_{P-O-C_2'-H} = 6.7$ Hz). No splittings can be resolved on the pyrophosphate signal, but from the line-broadening the couplings to the 5' protons must be small ($^3J_{P-O-C_5'-H_A} + ^3J_{P-O-C_5'-H_B} < 6$ Hz) (see also ref. 11).

The single-resonance ³¹P spectrum of the NADPH-enzyme complex (Fig. 1) suggests that the two phosphorus nuclei in the pyrophosphate are coupling to different extents to their corresponding 5' protons in the bound state. If reliable three-bond coupling constants could be measured this would enable us to determine the conformation about the C_{5'}-O_{5'} bonds in the bound coenzyme. Smith *et al.*¹² and others¹³⁻¹⁵, investigated the dependence of ³¹P-O-C-H coupling constants on the dihedral angle, θ , about the central bond. They found that there is a large difference in the magnitude of $^3J_{P-O-C-H}$ between *gauche* ($^3J_{P-O-C-H} = 1.8$ Hz) and *trans* ($^3J_{P-O-C-H} = 20.6$ Hz) conformations.

Unfortunately, the difference in linewidths between the ¹H noise-decoupled and single-resonance ³¹P spectra cannot be used directly to provide estimates of the coupling constants. A factor which must be considered in the measurement of coupling constants in large molecules is the possibility that rapid relaxation of one of the coupled nuclei is influencing the observed shape of the multiplets. Thus if the 5'CH₂ protons are rapidly relaxed then the measured coupling constants to the ³¹P nucleus could be smaller than the true coupling constants. The 5'CH₂ proton resonances cannot be observed in the ¹H spectrum of the coenzyme-enzyme complex which prevents us from measuring their *T*₂ values. By assuming a reasonable value for the correlation time of the complex, however, we have estimated that the *T*₂ values are in the range 0.02-0.03 s. Because of the dominant intramolecular contribution to the relaxation it is unlikely that the *T*₂ values for the two pairs of 5'CH₂ protons will be very different. We have calculated the effects of such relaxation rates on spin-multiplets with coupling constants in the range 1-20 Hz using the theory for the effects of exchange on line-shapes^{16,17}. Thus for the case of a doublet arising from coupling to such a relaxed proton when the coupling constant is 5 Hz the observed line broadening is ~ 1 Hz and for a coupling constant of 20 Hz the line broadening is ~ 16 Hz. For the high field pyrophosphate, the fact that we observe very little line broadening in the single resonance spectrum indicates that both P-O-C-H coupling constants are small (< 5 Hz); this of course assumes that our estimate that *T*₂ ≥ 0.02 s is correct. The lower field pyrophosphate shows considerable line broadening (~ 13 Hz) indicating that the sum of the coupling constants must be greater than 13 Hz (the existence of such large broadening shows that for these 5'CH₂ protons *T*₂ ≥ 0.02 s).

Ionisation state and conformation

The observation that the chemical shift of the 2'-phosphate ³¹P resonance is identical in the NADPH- and NADP⁺-reductase complexes clearly indicates that the 2'-phosphate group is binding in the same ionisation state and to the same site on the enzyme in both complexes. Since the chemical shift of this resonance is independent of pH within the range 4.5 to 7.5 in the complexes, the *pK* of the 2'-phosphate group must be

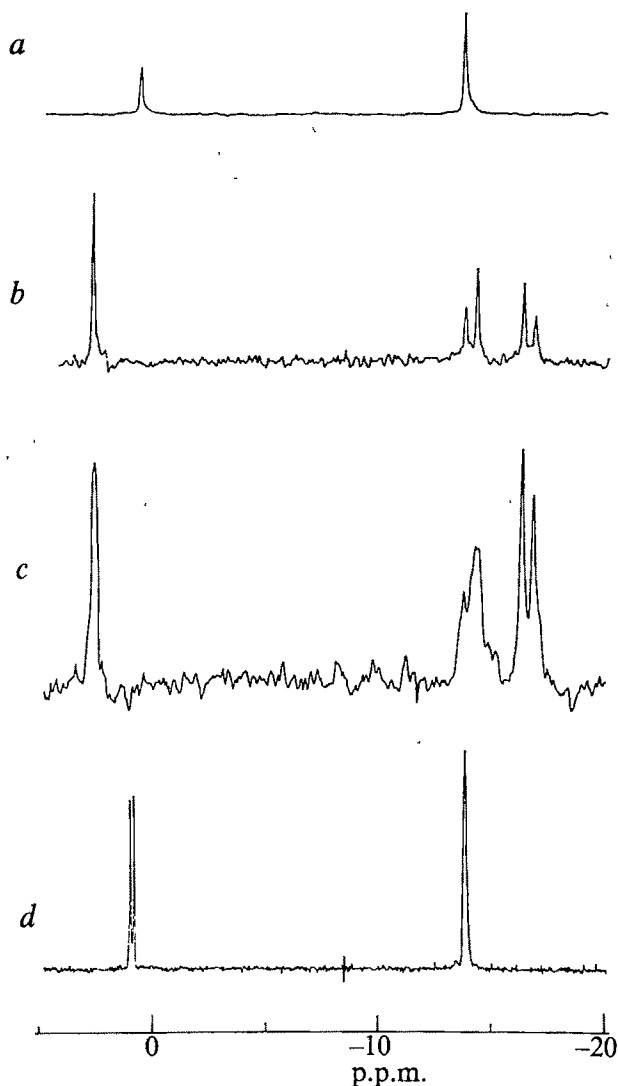


Fig. 1 a, The ³¹P proton noise decoupled spectrum of 10 mM NADPH at pH 6.9 (1,000 transients, 0.5 s acquisition time). b, The ³¹P proton noise decoupled spectrum of 1.3 mM NADPH in the presence of 1.3 mM *L. casei* dihydrofolate reductase at pH 6.9 (100 blocks of 1,000 transients 0.5 s acquisition time). c, ³¹P single resonance spectrum of 1.1 mM NADPH in the presence of 1.1 mM *L. casei* dihydrofolate reductase at pH 6.9 (319 blocks of 1,000 transients 0.5 s acquisition time). d, ³¹P single resonance spectrum of 50 mM NADPH at pH 7.9 (1,777 transients, 2.0 s acquisition time). All samples were dissolved in a D₂O buffer solution containing 500 mM KCl and 15 mM bis-Tris in the presence of 1 mM EDTA (except for sample used for spectrum (d) which contained 15 mM EDTA). The ³¹P Fourier transformed spectra were obtained at 40.5 MHz using a spectrometer (Varian XL-100) equipped with a computer (VDM 620 i). Exponential weighting functions of 0.1 s (spectrum c) and 0.2 s (spectra a and b) were used. The chemical shifts were externally referenced to 50 mM K₂HPO₄ solution (pH 8.0) contained in a capillary. The sample temperature was maintained at 11 ± 1 °C using a temperature unit V 4343.

at least three units different from its value in the free coenzyme. The position of the resonance (1.7 p.p.m. downfield from the dianionic and 4.5 p.p.m. downfield from the monoanionic form of the free coenzyme) suggests that the 2'-phosphate group binds in the dianionic form. This is confirmed by studies of the binding of 2'-AMP (B.B., J.F. and G.C.K.R., unpublished); for this compound the bound ^{31}P chemical shift is identical to that for the coenzyme complexes, and the binding constant decreases by more than a factor of ten on decreasing the pH from 7.0 to 5.5.

The decrease in pK of the 2'-phosphate group of the coenzyme by more than three pH units on binding to dihydrofolate reductase suggests that an electrostatic interaction between the phosphate group and a cationic group or groups makes a major contribution to the binding energy. Perhaps the most likely explanation of the additional downfield shift (1.7 p.p.m.) of the ^{31}P resonance from that expected for the dianionic ionisation state is that it is a linear electric field effect resulting from the proximity of a positively charged group on the enzyme. This interaction of the 2'-phosphate group presumably makes a major contribution to the hundred-

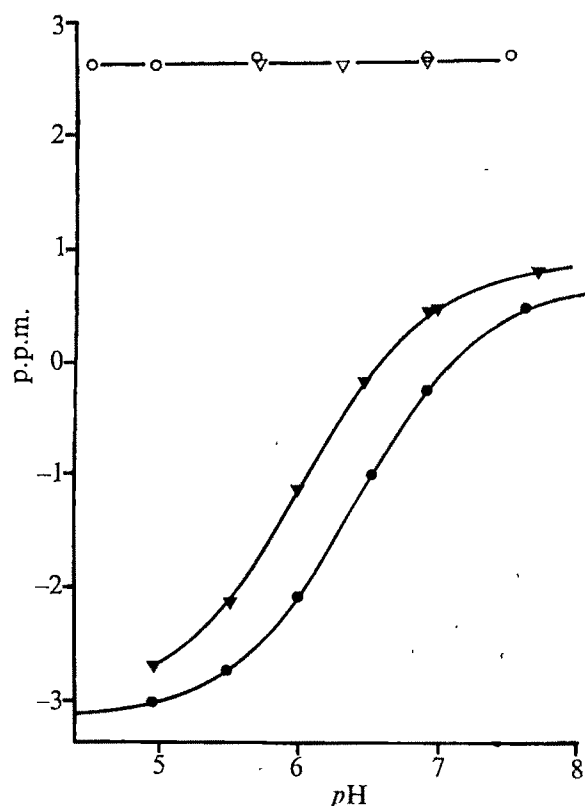


Fig. 2 Variation of chemical shift of the 2' phosphate ^{31}P resonance of NADP^+ (●, ○) and NADPH (▼, ▽) with pH. The open symbols refer to the coenzyme bound to dihydrofolate reductase and the solid symbols to the free coenzymes. The pH values are uncorrected meter readings (glass electrode) from D_2O solutions.

fold difference in the K_m values for NADPH and NADH seen for dihydrofolate reductase (J. Dann and G.C.K.R., unpublished).

The pattern of chemical shift changes for the two ^{31}P resonances of the pyrophosphate group is similar for both NADPH and NADP^+ complexes. One of the resonances is only slightly shifted in both cases; this and its $^3\text{J}_{\text{P-O-C-H}}$ coupling constants, indicate that this resonance represents the same phosphorus nucleus in both complexes. The other ^{31}P resonance shows a large upfield shift, which is significantly greater in the NADPH -enzyme complex than in the NADP^+ -enzyme complex. It is difficult to assess the significance of this difference, since we do not know the origin of the upfield shift on binding; it probably arises from a combination of

electric field effects and charge redistribution (perhaps accompanied by small changes in P-O-P angle¹⁸). ^1H NMR studies (our unpublished work) suggest that there may be conformational differences in the protein between the NADPH and NADP^+ complexes, but we cannot exclude the possibility that the difference in ^{31}P chemical shift is in some way a direct consequence of the positive charge on the nicotinamide ring of NADP^+ .

The narrow lines in the ^{31}P spectra of the coenzyme-dihydrofolate reductase complexes enabled us to measure $^{31}\text{P-O-C-}^{31}\text{P}$ spin-coupling and to observe the effects of $^{31}\text{P-O-C-}^{31}\text{H}$ spin-coupling. In experiments on other systems^{19,20}, coupling constants in the bound state were estimated by extrapolation for weakly binding ligands. The experiments described here, however, represent the first case in which direct coupling constant measurements have been possible for a ligand-protein complex.

The phosphorus nucleus giving rise to the highest-field signal shows small $^{31}\text{P-O-C-}^{31}\text{H}$ coupling constants (< 5 Hz), consistent only with a conformation in which it is *gauche* to both 5' protons. This is the conformation which is favoured for 5'-nucleotides²¹ and NADPH ¹¹ in solution. The other pyrophosphate phosphorus nucleus, which shows the smaller shift, shows a larger change in conformation differing from the *gauche-gauche* by at least 50° (based on considerations of the Karplus equation of Smith *et al.*¹²).

The conformations of NAD bound to lactate dehydrogenase²² and of ADP-ribose bound to alcohol dehydrogenase²³ are known from crystallographic studies. In both cases, the conformation about both $\text{C}_5'-\text{O}_5'$ bonds is *gauche-gauche*. For these enzymes, therefore, in contrast to dihydrofolate reductase, the binding of coenzyme is not accompanied by any change in the conformation about the $\text{C}_5'-\text{O}_5'$ bonds. At present, we are not able to specify which of the two $\text{C}_5'-\text{O}_5'$ bonds of NADPH shows a change in conformation on binding to dihydrofolate reductase, since we cannot yet assign the two pyrophosphate ^{31}P resonances in the complex. In view of the structural similarities in the coenzyme binding sites of a number of dehydrogenases^{24,25}, a comparison of the conformation of the bound coenzyme in other dehydrogenases will be of considerable interest, and we are attempting to extend our ^{31}P NMR studies in this direction, as well as studying the effects of substrate and inhibitor binding to dihydrofolate reductase on coenzyme conformation.

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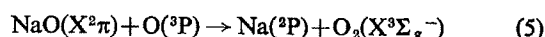
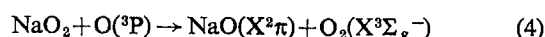
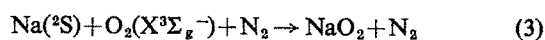
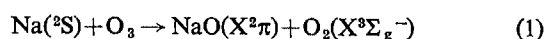
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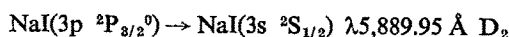
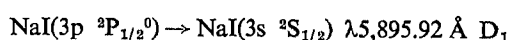
Sodium emission from long enduring meteor trains

ONE of the more perplexing atmospheric emission phenomena is the occurrence of long enduring visual meteor trains which, on rare occasions, are observed to persist for about 1 h. The mechanism responsible for the long lived luminosity is, however, elusive. Chapman¹ suggested that a source of night-time train luminosity is to be found in the store of recombination energy of free atmospheric atomic oxygen. He envisaged the role of Na atoms as continuously catalysing the transformation of atomic oxygen to molecular oxygen by sodium monoxide (NaO) formation so that the dissociation energy of O₂ was converted into sodium light with the aid of the atmospheric species O₃ and O₂. Here I examine the energetics of the sodium process and estimate the associated train luminosity.

The complete sequence of reactions is,



where the specific transitions are



At meteoric heights the atom interchange of NaO and O₃ to form NaO₂ or Na will be negligible.

When Chapman made his proposal the energetics of the various processes were not accurately known and, in particular, the exothermicity of reaction (5) was in doubt. Uncertainties arose because of the difficulties of measuring the dissociation energy, $D_0^0(\text{NaO})$, of the NaO molecule in the laboratory. Since the exothermicity of reaction (5) for producing the ground state sodium atom, Na(²S), is given by $D_0^0(\text{O}_2) - D_0^0(\text{NaO})$, then with $D_0^0(\text{O}_2) = 5.11 \text{ eV}$ the bond energy of NaO for the production of excited Na(²P) (2.10 eV) must be $D_0^0(\text{NaO}) < 66.2 \text{ kcalorie mol}^{-1}$ (3.0 eV). Although early studies indicated a lower limit to $D_0^0(\text{NaO})$ of 72 kcalorie mol⁻¹, theoretical and experimental work² has confirmed the value $60.3 \pm 4.0 \text{ kcalorie mol}^{-1}$, corresponding to $2.60 \pm 0.2 \text{ eV}$, making reaction (5) exothermic by $0.4 \pm 0.2 \text{ eV}$. The branching ratio of reaction (5) to yield ground state Na(²S) or excited Na(²P) is unknown.

Using the model that a solid meteoroid of mass $\sim 30 \text{ g}$ and velocity 40 km s^{-1} will produce a meteor with visual magnitude of about -5 , published ablation data³ can be extrapolated such that at zenith angle 30° the meteoroid will deposit meteoric atoms of linear density $\sim 4 \times 10^{17} \text{ cm}^{-1}$. With an

atomic abundance for Na of 0.5% (ref. 4) the line density of deposited Na atoms becomes $\alpha_{\text{Na}} = 2 \times 10^{15} \text{ cm}^{-1}$. The fraction of these lost in forming Na⁺ by collisional ionisation is small at the velocity of meteors.

Extensive observational data show that the heights of long enduring visual trains are confined to 85–90 km. With a molecular diffusion coefficient at the lower height for Na atoms of $4.2 \text{ m}^2 \text{ s}^{-1}$ and a radius, r , at time t of the radially expanding Na column given by $r^2 = 4Dt$ such a train will remain unresolved in a transverse direction by the naked eye for about 20 min. In such circumstances in the absence of turbulence and for an optically thin medium, the light intensity I is simply the sum of the quanta from all excited Na atoms in a cross section of the meteor train of unit length. Not only will atmospheric atomic oxygen of number density $[\text{O}_a]$ take part in the various reactions but also meteoric atomic oxygen of density $[\text{O}_m]$. With a meteoroid oxygen abundance of about 50%, then for a -5 mag meteor $\alpha_{\text{O}} = 2.5 \times 10^{17} \text{ cm}^{-1}$. For ease of computation let all species have a common diffusion coefficient and treat atmospheric oxygen as a sink, then the set of equations describing the number densities of species may be written (representing, for convenience, number densities simply by the species symbol)

$$\frac{\partial}{\partial t} \text{Na} = D\nabla^2 \text{Na} + k_5 \cdot \text{NaO} \cdot (\text{O}_a + \text{O}_m) - k_1 \cdot \text{Na} \cdot \text{O}_3 - k_3 \cdot \text{Na} \cdot \text{O}_2 \cdot \text{N}_2 - k_2 \cdot \text{Na} \cdot (\text{O}_a + \text{O}_m) \cdot \text{N}_2$$

$$\frac{\partial}{\partial t} \text{NaO} = D\nabla^2 \text{NaO} + k_1 \cdot \text{Na} \cdot \text{O}_3 + k_2 \cdot \text{Na} \cdot (\text{O}_a + \text{O}_m) \cdot \text{N}_2 + k_4 \cdot \text{NaO}_2 \cdot (\text{O}_a + \text{O}_m) - k_5 \cdot \text{NaO} \cdot (\text{O}_a + \text{O}_m)$$

$$\frac{\partial}{\partial t} \text{NaO}_2 = D\nabla^2 \text{NaO}_2 - k_3 \cdot \text{Na} \cdot \text{O}_2 \cdot \text{N}_2 - k_4 \cdot \text{NaO}_2 \cdot (\text{O}_a + \text{O}_m)$$

$$\frac{\partial}{\partial t} \text{O}_m = D\nabla^2 \text{O}_m - k_2 \cdot \text{Na} \cdot \text{O}_m \cdot \text{N}_2 - k_4 \cdot \text{NaO}_2 \cdot \text{O}_m - k_5 \cdot \text{NaO} \cdot \text{O}_m$$

$$I = \Sigma d/dt [\text{Na}(^2\text{P})] = \Sigma k_5 \cdot f \cdot \text{NaO} \cdot (\text{O}_a + \text{O}_m) \text{ photons s}^{-1} \text{ cm}^{-1}$$

where ∇^2 is the Laplacian operator in cylindrical coordinates, and an initial meteor train radius of 1 m is assumed. Such a set may be solved using an implicit finite difference scheme⁵. Na atoms are continuously cycled and no overall night-time chemical loss of Na is included. The loss-lifetime from Na⁺ formation due to charge exchange with atmospheric O₂⁺ and NO⁺ will be of the order of 10⁵ s. Some initial charge transfer of Na atoms with ions of atoms with higher ionisation potential (such as Si, Fe, Mg) might be expected. Since the initial metal ion abundances are, however, generally rather less than those of Na atoms, no significant loss will occur.

Of the reactions (1)–(5) only the three-body association (reaction (3)) seems to have been studied in the laboratory and thus is the only one for which direct measurements of rate coefficient are available. For $T = 200 \text{ K}$, and height = 85–90 km we adopt (from ref. 6) $k_3 = 2.0 \times 10^{-32} \text{ cm}^6 \text{ s}^{-1}$. Rate coefficients which appear in the literature for the other reactions are based on the values for similar processes involving N and H and we use⁶ the following; $k_1 = 10^{-13} \text{ cm}^3 \text{ s}^{-1}$, $k_2 = 10^{-33} \text{ cm}^6 \text{ s}^{-1}$, $k_4 = k_5 = 10^{-11} \text{ cm}^3 \text{ s}^{-1}$. Uncertainties of a factor of three might be expected in all rate coefficients. The rate at which

the whole chemical cycle proceeds is determined mainly by reactions (1) and (3) and therefore in part by the concentration of the minor atmospheric species, O_3 . Using the first direct mass spectrometer measurements⁷ (obtained during early evening) we used densities of 8×10^8 and $3 \times 10^8 \text{ cm}^{-3}$ at 85 and 90 km respectively. The night-time densities of oxygen used are $3 \times 10^{11} \text{ cm}^{-3}$ at 90 km, and $7 \times 10^{10} \text{ cm}^{-3}$ at 85 km. The main gas densities (cm^{-3}) used are $[N_2] = 1.2 \times 10^{14}$ and $5.0 \times 10^{13} \text{ cm}^{-3}$ at 85 and 90 km, and $[O_2] = 3.2 \times 10^{13}$ and $1.3 \times 10^{13} \text{ cm}^{-3}$ at 85 and 90 km respectively. Solutions of the set of diffusion equations were computed (see for example Fig. 1). The emission for $t < 0.1 \text{ s}$ is caused by the effect of the large initial meteoric O_m concentrations and the associated emission decay by the subsequent rapid diffusion of O_m . For times up to

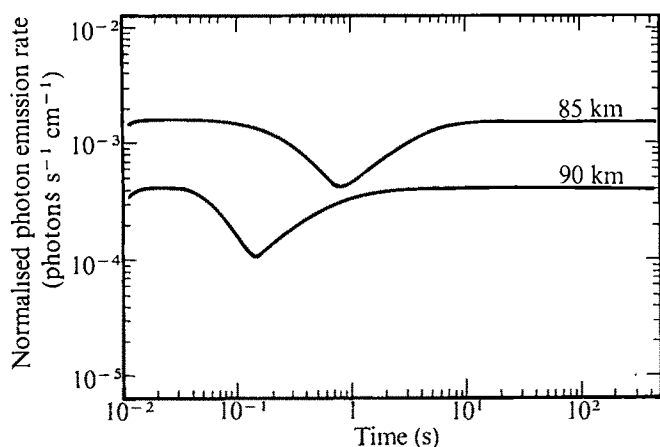


Fig 1 Solutions for the case of a meteor of approximately -5 mag occurring at heights of 85 and 90 km. Branching factor $f = 0.2$. Ordinate is the photon emission rate, I , normalised to the value of $4 \times 10^{13} \text{ photons s}^{-1} \text{ cm}^{-1}$. Parameters used are: $\alpha_{Na} = 2 \times 10^{15} \text{ cm}^{-1}$, $\alpha_{O_m} = 2.5 \times 10^{17} \text{ cm}^{-1}$, $D_{85} = 4.2 \text{ m}^2 \text{ s}^{-1}$, $D_{90} = 10 \text{ m}^2 \text{ s}^{-1}$. An initial neutral atom radius of 1 m assumed at both heights.

several hundred seconds while the train remains unresolved the computed emission rates are 6.4×10^{10} and $1.7 \times 10^{10} \text{ photons s}^{-1} \text{ cm}^{-1}$ at 85 and 90 km respectively. The first point is how this emission compares with the observed train luminosities. Taking the estimate⁸ of the minimum detectable emission for the naked eye as $30 \text{ erg s}^{-1} \text{ cm}^{-1}$ and assuming that a clearly visible train is 2 mag brighter than this limit, the required emission rate in the yellow is $\sim 5 \times 10^{13} \text{ photons s}^{-1} \text{ cm}^{-1}$, indicating that the computed emissions for a -5 mag meteor fail by 7.0 and 8.3 mag. We infer that meteors of -12.0 and -13.3 mag could produce the required luminosity for 85 and 90 km respectively. The second important point is how the frequency of occurrence of such bright meteors compares with that of long duration trains. Since about 1 in 10^3 visual meteors produces a train of duration exceeding 10 s and with the observation that of those night-time trains of durations greater than 10 s in the extensive records of Olivier⁹ 1.5% had durations in excess of 30 min, then, with an observed visual rate of about 10 h^{-1} , it is inferred that the frequency of occurrence of trains longer than 30 min for a single observer is about $1.5 \times 10^{-6} \text{ h}^{-1}$. In comparison, the observed integrated flux¹⁰ of bright meteors indicates that the occurrence frequencies of meteors brighter than -12.0 and -13.3 for a single observer are 1.2×10^{-7} and $2.2 \times 10^{-8} \text{ h}^{-1}$ respectively.

If every sodium oxide reduction reaction produces an Na atom in an excited 3P state ($f = 1.0$) then at 85 km a meteor of about -10.25 mag is required to yield sufficient train luminosity. Though such bright objects certainly do produce long enduring trains⁹ the occurrence frequency of meteors brighter

than this limit is a factor of 13 smaller than the observed frequency of trains of duration in excess of 30 min. A large proportion of trains reported⁹, however, occurred during major showers particularly the Perseids and Leonids when the bright meteor flux is several times larger than the sporadic flux.

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Observations of eight globular clusters at 2.3 and 4.7 μm

THE Cerro Tololo infrared photometer¹ has been used to search several globular clusters for infrared emission between 2.3 and 20 μm , principally to seek evidence for the presence of dust. Recently, more extensive observations using improved short wavelength instrumentation have been obtained at 2.3 and 4.7 μm . The latter observations were concentrated on two groups of predominantly southern clusters: (1) two globular clusters (NGC 6388 and 6441) rich in metals and suggested² to be of particular interest for the detection of gas thought to be lost from stars during the normal course of stellar evolution; and (2) six clusters with short core relaxation times and large central densities discussed³ in connection with the possible presence of massive central black holes in clusters associated with X-ray sources.

The observations at 2.3 and 4.7 μm (with bandwidths of 0.37 and 0.60 μm , respectively) discussed here were obtained on two nights in June, 1975 using the 1.5-m telescope on Cerro Tololo and an InSb detector on the dual-beamed photometer which operates at 10 Hz with an 80% duty cycle. Each cluster was carefully centred visually in the $10''$ aperture, after which precalculated offsets to the edge of the cluster were used to fix the starting point for a series of diametric drift scans. Typically, 40 drift scans in right ascension per cluster at each wavelength were obtained. As the fixed separation of $28.5''$ on the sky between the two beams (normally the 'star' and 'sky' positions) of the photometer was less than the cluster diameter, the drift scan technique actually measures an intensity gradient, and data reduction was performed on this basis. The integration time was set to equal the time to drift the beam separation distance; each drift scan therefore can be thought of as a number of discrete steps.

Table 1 lists the clusters observed, the number of scans obtained at each wavelength, the steps per scan, the total integration time per step, and the approximate apparent magnitude of each object. As each spatially resolvable point was effectively observed for only a relatively short time ($\sim 100 \text{ s}$), the detection threshold of the measurements is not as low as could be achieved by observing a single cluster-sky-point pair for a longer time. But, as one of the largest uncertainties in the theoretical^{2,3} and observational (refs 4 and 5 and M. G. Smith, J.E.H. and S. J. Shawl, unpublished) work done to date on the presence of gas and dust in globular clusters relates to the volume over which one might expect to

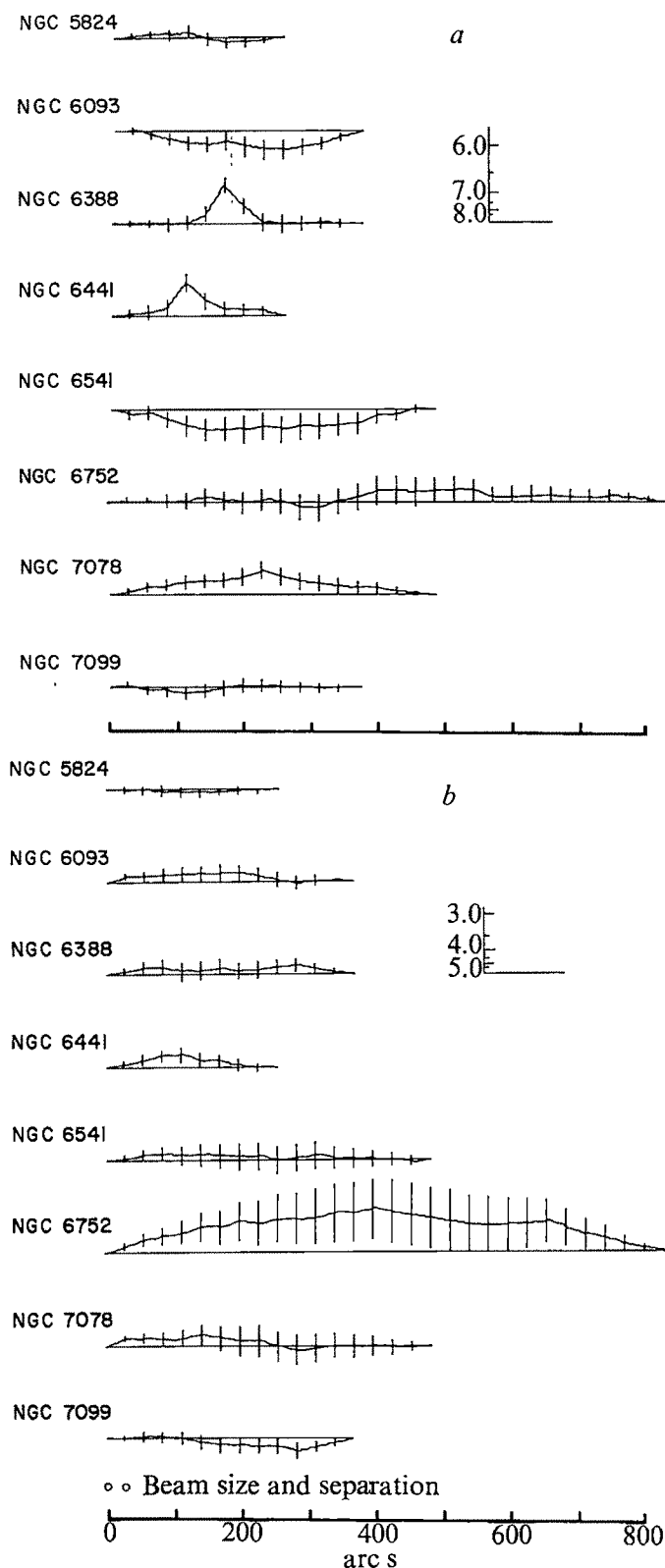


Fig. 1 Average, rectified drift scans of 8 globular clusters at 2.3 (a) and 4.7 μm (b), respectively. Error bars indicate the r.m.s. deviation among individual scans. Each point is treated separately, that is point-to-point coherence has not been invoked. The endpoints of each individual scan have been assumed to be zero; the error bars therefore decrease artificially towards both endpoints. The magnitude scale inserted in the upper right-hand corner of each figure refers to height above the baseline for a uniform brightness distribution across the 10'' aperture.

find any such interstellar material, drift scanning offered certain advantages for a pilot study such as this.

From these observations, shown in detail in Fig. 1a and b, we find that the central regions of the two massive, metal-rich clusters NGC 6388 and 6441 (refs 6 and 7) were detected at 2.3 μm but probably not at 4.7 μm. Taking the errors into consideration, the amplitude of the emission may plausibly be attributed entirely to the giants contained within the sampled volume, provided the luminosity function tabulated by Allen⁸ and the core and tidal radii derived by Illingworth⁶ and Peterson and King⁹ are applicable. Similarly, the failure to detect significant radiation at 4.7 μm is consistent with the 2.3-μm flux being due to radiation characterised by $T_{\text{eff}} > 1,000$ K. As dust would probably be cooler, the observed radiation is more likely of stellar origin. With the possible exception of NGC 7078 (M 15), all other clusters in Fig. 1 and Table 1 (which are those in the upper right-hand portion of Bahcall and Ostriker's³ figure), yield null results within the limits of this study.

Earlier observations at 2.3, 4.7, 10 and 20 μm of NGC 104 (47 Tucanae), 1851, 2808 and 5139 (ω Centauri) failed to show any emission, but the detection limits were considerably less significant than those of the more recent short wavelength observations and they have not been included here. Clearly, important clusters such as NGC 104 (massive and metal rich^{6,10}) and NGC 1851 (a transient X-ray source¹¹) deserve much closer future attention, as do NGC 6388 and 6441.

Finally, should the intriguing observation of a 10-μm emission source in NGC 7078 (M 15) (refs 12 and 13) be confirmed, the present observations may be used to set limits on the (2.3–10) and (4.7–10)-μm colour indices of the emitter.

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Table 1 Observational data

NGC	Scans	Steps per scan	2.3 μm		Scans	Steps per scan	4.7 μm	
			Time per step(s)	Apparent magnitude			Time per step(s)	Apparent magnitude
5824	40	10	91	> 8.8	44	10	100	> 5.5
6093	46	14	95	> 9.0	47	14	97	> 5.1
6388	38	14	102	> 6.7	38	14	102	> 5.1
6441	42	10	100	> 6.9	43	10	102	> 4.7
6541	32	18	84	> 9	33	18	87	> 5.0
6752	25	30	95	> 7.9	25	30	95	> 3.4
7078	51	18	99	> 7.2	49	18	95	> 4.8
7099	49	14	101	> 9	54	14	112	> 5.5

Palaeomagnetic evidence from DSDP cores of northward drift of India

EVIDENCE of latitudinal changes associated with the northward movement of India during the Cainozoic have been described¹ from remanence inclination measurements made on sediments and basalts recovered at DSDP sites in the Arabian Sea (Fig. 1). When combined with palaeomagnetic data from continental India²⁻⁴ the results showed an initial northward movement at a mean rate of 26 cm yr⁻¹ from the Cretaceous to at least Middle Eocene times and a subsequent slower mean rate of 16 cm yr⁻¹ being resumed in the Miocene (Fig. 2a). The original analysis implied a possible pause in motion between the two phases during the Oligocene, for which period no data were available.

Further palaeomagnetic measurements have been completed by us following the recovery during Leg 24 (ref. 5) of Oligocene sediments and basalts at Deep Sea Drilling Project (DSDP) site 238 on the southern edge of the Indian Plate (Fig. 1). Only those samples considered the most stable after demagnetisation are included in the inclination values in Table 1. Palaeolatitudes from site 238 do not seem to support the phase of rapid post-Middle Miocene plate motion (Fig. 2b) but indicate instead that India may have already been much closer to its present latitudinal position by the early Oligocene.

There are several possible reasons for the discrepancy in Oligocene and post-Oligocene palaeolatitudes.

Non-verticality of coring. Inclination measurements at several sites during DSDP Leg 25 (ref. 6) indicate that the holes were never more than 5° off the vertical. Assuming a similar near verticality for the DSDP sites discussed here, errors in palaeolatitudes (probably a maximum of 2–3°) associated with non-vertical holes are, therefore, inadequate to account for the observed differences between Oligocene and post-Oligocene palaeolatitudes. There is no positive indication that DSDP site 238 has been subjected to any appreciable post-depositional tectonic tilting which would affect the observed remanent inclinations. All visible bedding relationships throughout the lower part of the core are horizontal or near horizontal.

Averaged geomagnetic field configuration other than a geocentric axial dipole. All palaeolatitudes considered so far have been calculated from mean absolute inclination values assuming a geocentric axial dipole approximation ($\tan \lambda = \frac{1}{2} \tan I$). Wilson⁷ considers, however, that the geomagnetic field for the Quaternary and Tertiary (as far back as the Miocene) may be better described by an offset dipole, the northward displacement of the dipole during these periods being 191 ± 38 and 306 ± 41 km respectively. All calculated palaeolatitudes younger than the Miocene may therefore be too far south by up to 4° as a result of this offset. Palaeolatitudes have been recalculated from mean absolute inclinations assuming a displaced axial dipole field since the Miocene (Fig. 2c). A rapid though somewhat decreased drift is still apparent for the post-Oligocene sites, requiring a 15° latitudinal change since Middle Miocene times. The offset dipole model then may account for some of the observed disagreement but the revised palaeolatitudes are still broadly inconsistent with the data from DSDP site 238.

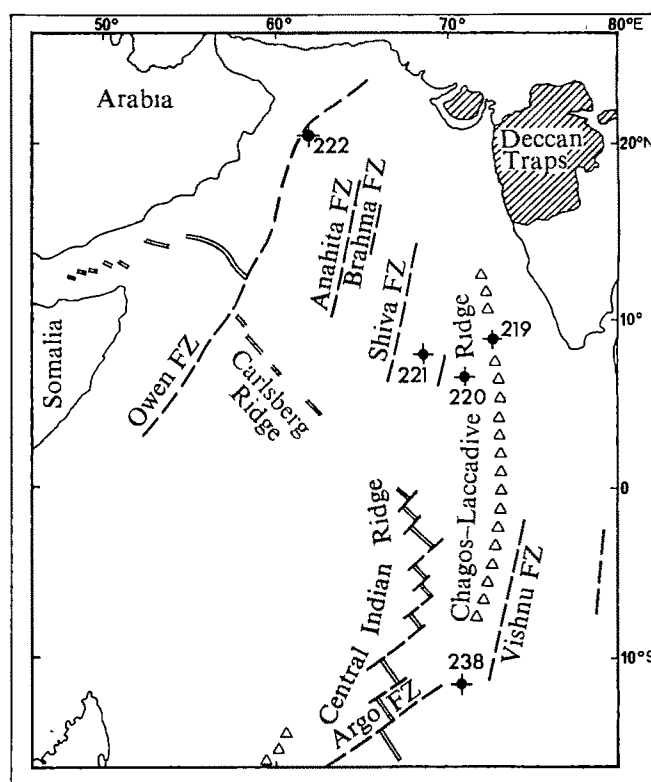


Fig. 1 Location of DSDP site 238 and other DSDP sites in the Arabian Sea which lie on the Indian Plate. FZ, Fracture zone.

Primary remanence deviations. Opdyke and Henry⁸ showed that remanence inclination errors⁹ were not present in some 52 deep-sea sediment cores. The sediments of those cores are, however, predominantly slowly accumulated pelagic and hemipelagic sediments and biogenic oozes for which average sedimentation rates of 10 m Myr⁻¹ are typical¹⁰. For current deposited deep-sea sediments, containing a predominantly terrigenous component and characterised by much faster sedimentation rates, significant inclination errors have been recorded^{11,12}. The mode of acquisition of depositional remanent magnetisation (DRM) is not yet fully understood but a shallowing of inclination might be expected due to gravitational couples (a true inclination error as defined by King⁹), or hydrodynamic tangential shearing of remanence carriers on deposition (rotation effect¹³), to post-depositional compaction, or to any combination of these. Further detailed analysis of the mean inclinations exhibited by terrigenous sediments is required to confirm the presence of such primary remanence deviations. If the possibility of significant inclination errors being associated with terrigenous sediments is acknowledged then the effect of such errors on the DSDP sediments reported here can be considered.

Sediments from DSDP sites 238 (nannochalk oozes, sedimentation rate = 12.7 m Myr⁻¹)⁵, 220 (laminated chalks, sedimentation rate = 19 m Myr⁻¹)¹⁴, and the Pliocene–Miocene sequence from DSDP site 221 (pelagic brown clay,

Table 1 Palaeomagnetic results for DSDP site 238

Site	Material	No. of samples	Age	Mean absolute inclination	Observed palaeolatitude	Reduced palaeolatitude*
238	Sediments	22	Early Miocene–late Oligocene 19–30 Myr	$30.5 \pm 3.2^\circ$	$16.4 \pm 2.0^\circ\text{S}$	$3.7 \pm 2.0^\circ\text{N}$
	Basalts	48	Early Oligocene 34–38 Myr	$28.2 \pm 1.0^\circ$	$15.0 \pm 0.6^\circ\text{S}$	$5.1 \pm 0.6^\circ\text{N}$

All errors are s.e.

*Palaeolatitudes reduced to the common latitude of DSDP site 219 according to the method of Whitmarsh *et al.*¹.

Table 2 Inclination error corrections and corresponding reduced palaeolatitudes

Site	Age	Mean inclination	Inclination error	Corrected palaeolatitude	Reduced corrected palaeolatitude
219	Upper Palaeocene	$29.1 \pm 5.6^\circ$	20.1°	$30.1 \pm 5.7^\circ\text{S}$	$30.1 \pm 5.7^\circ\text{S}$
221	Pleistocene	$15.2 \pm 5.9^\circ$	14.3°	$15.8 \pm 6.1^\circ\text{N}$	$16.8 \pm 6.1^\circ\text{N}$
222	Lower Pliocene	$22.1 \pm 4.9^\circ$	18.1°	$22.9 \pm 5.1^\circ\text{N}$	$11.8 \pm 5.1^\circ\text{N}$
Lower Siwaliks	Upper Miocene	$18.8 \pm 1.7^\circ$	16.5°	$19.5 \pm 1.8^\circ\text{N}$	$8.4 \pm 1.8^\circ\text{N}$
	Middle Miocene	$28.1 \pm 2.1^\circ$	19.9°	$29.1 \pm 2.1^\circ\text{N}$	$5.3 \pm 2.1^\circ\text{N}$

sedimentation rate = 2 m Myr^{-1})¹⁴ are of similar lithology and have sedimentation rates comparable with those sediments described above which are known to record accurately the ambient field inclination value at the time of sediment formation: mean inclinations from these sites thus provide a reliable estimate of site palaeolatitude during the corresponding stratigraphic intervals. In contrast, sediments sampled for palaeomagnetic study at DSDP sites 222 (grey detrital silty clays, sedimentation rate = $135\text{--}600 \text{ m Myr}^{-1}$)¹⁴, 219 (sandstones and siltstones, sedimentation rate $> 70 \text{ m Myr}^{-1}$)¹⁴ and the late Pliocene-late Pleistocene turbidite sequence from DSDP site 221 (silt, sedimentation rate = 170 m Myr^{-1})¹⁴ are all rapidly deposited terrigenous sediments with which significant inclination errors may be associated. An initial estimate of the magnitude of such inclination errors may be obtained by a consideration of the true inclination error predicted by the rolling spheres model¹⁵. The mean observed inclination (I_0) is related to mean geomagnetic field inclination (I_f) by the expression¹⁶:

$$\tan I_0 = 0.48 \tan I_f$$

from which the computed value of I_f has been used to calculate a revised palaeolatitude, assuming again a geocentric axial dipole model. Inclination error corrections, where applicable, together with the revised palaeolatitudes are given in Table 2. The directions of magnetisation of the Lower Siwalik detrital red shales are considered to have been acquired during deposition²: accepting a DRM origin for these rapidly deposited¹⁷ continental sediments an inclination error correction has been applied as above. All corrected site palaeolatitudes again reduced to the common latitude of DSDP site 219 are shown plotted in Fig. 2d. Allowance for inclination error effects may be seen to improve markedly the internal agreement of the palaeolatitude data from these DSDP cores. A possible overestimation of inclination errors in natural sediments by the rolling spheres model may account for the more northerly placing of the palaeolatitudes of DSDP sites 221 and 222 than is expected from their present site latitudes.

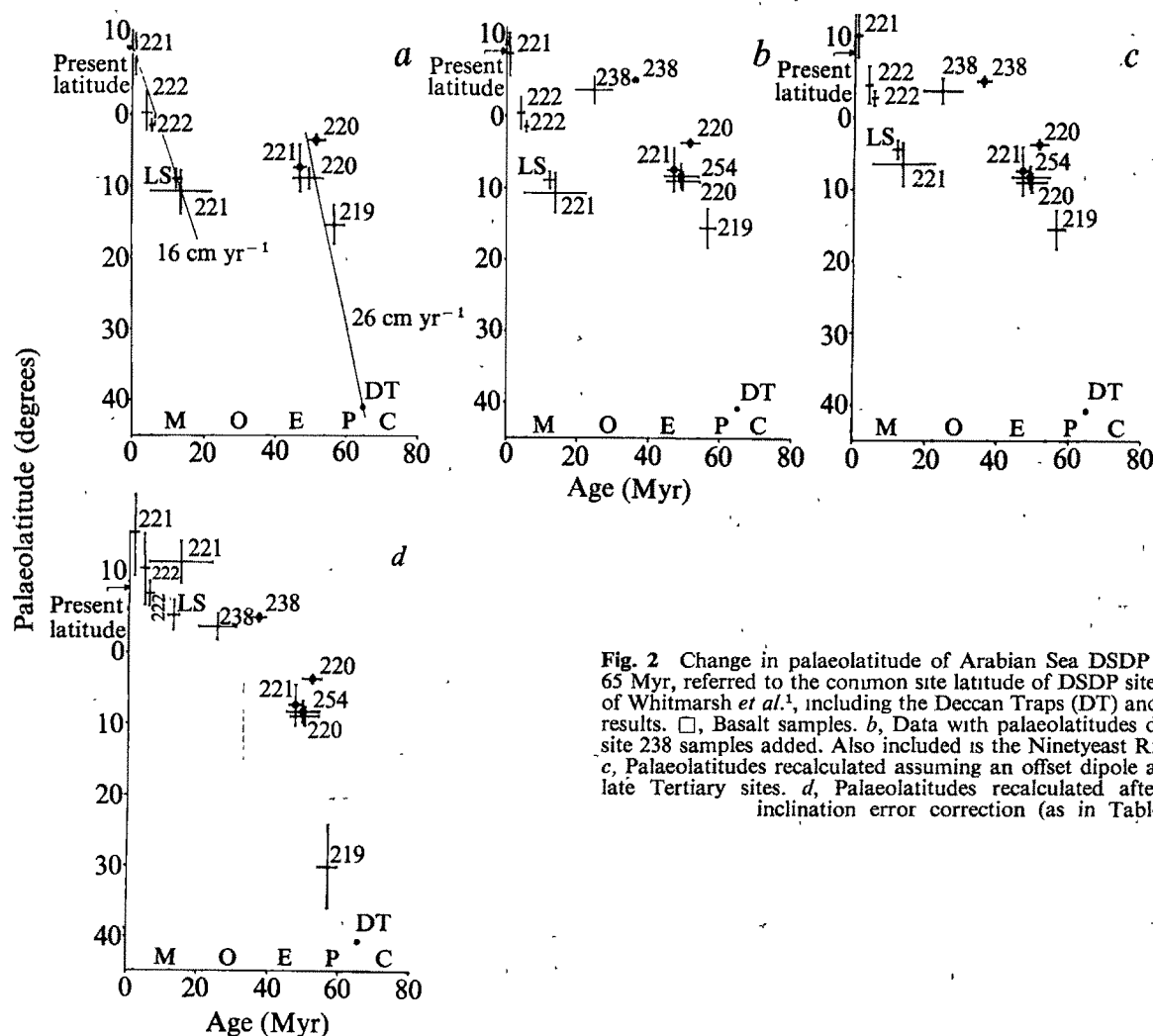


Fig. 2 Change in palaeolatitude of Arabian Sea DSDP sites during the past 65 Myr, referred to the common site latitude of DSDP site 219. *a*, Original data of Whitmarsh *et al.*¹, including the Deccan Traps (DT) and Lower Siwaliks (LS) results. □, Basalt samples. *b*, Data with palaeolatitudes determined for DSDP site 238 samples added. Also included is the Ninetyeast Ridge site 254 (ref. 20). *c*, Palaeolatitudes recalculated assuming an offset dipole approximation for the late Tertiary sites. *d*, Palaeolatitudes recalculated after application of an inclination error correction (as in Table 2).

The revised northward drift history of the Indian plate during the late Tertiary indicated by the DSDP cores described here is broadly in agreement with the Australian palaeomagnetic data^{18,19}. It provides an opportunity to qualify further the time at which the early Tertiary phase of rapid spreading ceased and the nature of the motion since that time. From the palaeomagnetic standpoint this assessment of palaeolatitudes has indicated that inclination errors may yet be of importance in certain clastic sedimentary formations.

We thank Dr R. B. Whitmarsh for his continued interest and comments. The samples were made available through the assistance of the Deep Sea Drilling Project and the US National Science Foundation. Part of this study was completed during the tenure of an NERC research studentship to R.A.B.

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Seismic evidence for local undulation of olivine-spinel phase boundary

A DETAILED P-wave velocity distribution has been determined (S.D.-S. and R.A.W., unpublished) for the upper mantle in western Canada. The analysis was based on differential travel-time and relative-amplitude observations of multiple arrivals for seismograms between 14 and 40°. Three velocity models were determined for three different regions. All of these models are characterised by discontinuities at 410 and 650 km. The reflection from the 410-km discontinuity is best observed between 14 and 18°. While analysing seismograms between 14 and 18°, a group of the 410-km discontinuity. Here we present these observations and give a speculative explanation for the undulation of 410-km discontinuity. Here we present these observations and give a speculative explanation for the undulation.

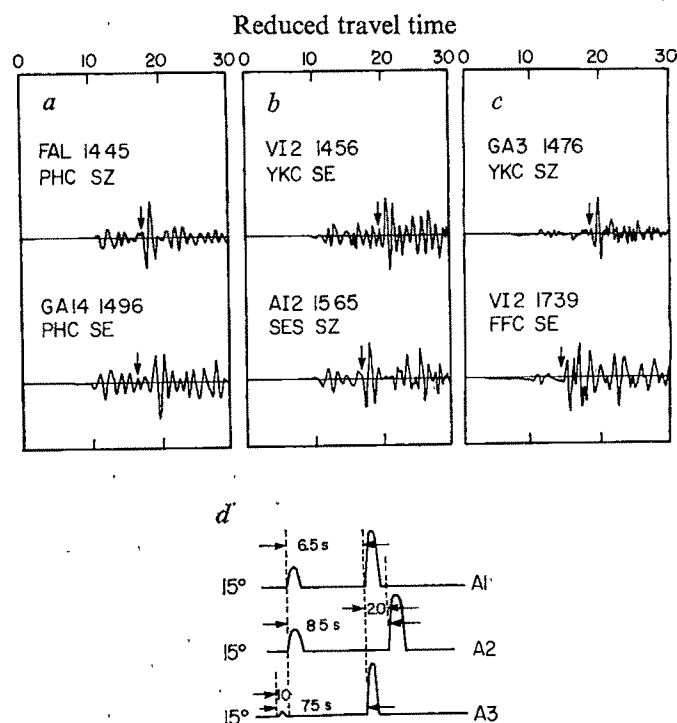


Fig. 1 Some typical seismograms of types A1 (a), A2 (b) and A3 (c) in group A (14–18°). d, Schematic representation at 15°. FAL, Faultless; VI, Vancouver Island; GA, Gulf of Alaska; AI, Alaskan Islands.

Figure 1 shows some typical seismograms around 15°, designated as group A type and subdivided into, A1, A2 and A3. The abbreviated event name and the epicentre distance are written at the top, the station name and the component of motion are written below. The name of the event roughly corresponds to its geographical location. The first arrival is always aligned at the 10.0 mark. Figure 2 shows the spatial locations of all observations. Each arrow represents the source station midpoint and the direction of the arrow indicates the direction in which the ray is travelling at its turning point. This midpoint represents the surface projection of the turning point of the ray at depth.

The amplitude ratio of second to first arrival is about 3 : 1 in A1 (Fig. 1a) and A2 (Fig. 1b) type records, in contrast to about 10 : 1 in A3 (Fig. 1c) type records. This difference in relative amplitude behaviour between A1 and A3 records was responsible for the two velocity models WCA and WCB. In model WCB, the velocity at the base of the low velocity zone is 8.4 km s⁻¹ compared with 8.17 km s⁻¹ in model WCA. This causes the first arrival to arrive about 1 s early between 14 and 18° with respect to that in WCA. As can be seen from Fig. 2, model WCA is valid for the ocean and the region near the ocean-continent boundary, whereas model WCB is valid for regions east of the Rocky Mountain trench.

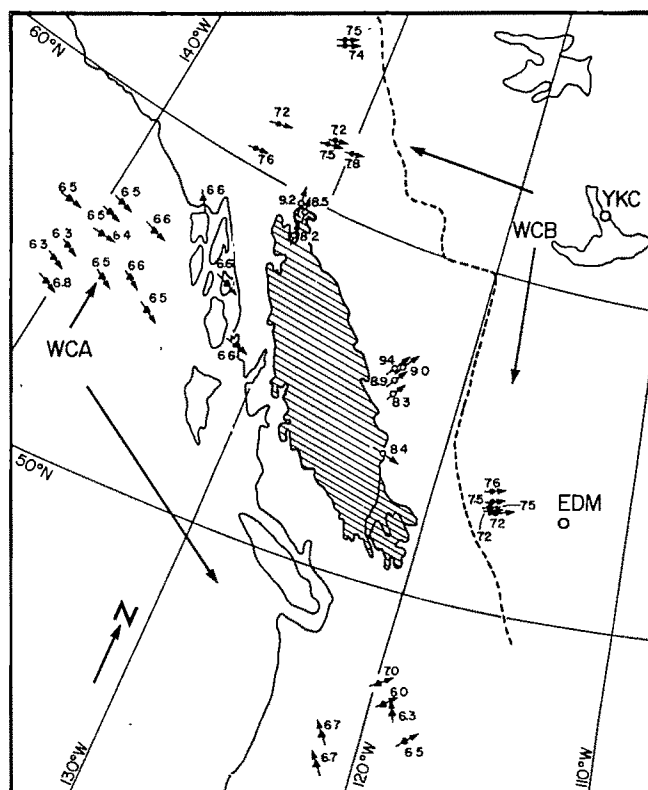
The A2 type seismograms, which are similar to A1 types as far as relative amplitude is concerned, are observed

Table 1 First arrival time data for A1 and A2 seismograms for distances around 15°

Event	Station	Distance (°)	Travel time (s)	Reduced travel time at 15° (s)	Mean (s)	Seismogram type
VI3	YKC	14.81	210.0	212.40	211.5	A2
VI4	YKC	14.97	210.5	210.90		A2
VI5	YKC	15.05	212.0	211.40		A2
Faultless	PHC	14.45	204.0	210.90	211.7	A1
Handley	PHC	15.52	217.0	210.50		A1
GA14	FSJ	14.60	208.5	213.50		A1
GA14	PHC	14.96	211.6	212.10		A1

VI, Vancouver Island; GA, Gulf of Alaska.

In Fig. 2, the hatched area represents the region of Cainozoic and Mesozoic volcanic activity along with some sedimentary formations. The location of downbuckling is very close to this large scale volcanic activity. The volcanics are of the alkali-olivine type³, where olivine is generally more rich in iron than in magnesium⁴. If by localised partial melting at a depth of 410 km the olivine is enriched in magnesium with respect to the surrounding region, then it will require a higher pressure to achieve the phase transformation to spinel. Thus the discontinuity will show a local depression. This partial melting of the olivine will then generate the iron-rich alkali-olivine basalts observed on the surface. The obvious flaw in this argu-



(IOKAE) Here we present the first sedimentary province

A PRELIMINARY survey of present-day sediment distribution within the Saronic Gulf system, off the coast of Greece, has been made in support of an ongoing study being conducted by the Institute of Oceanographic and Fishing Research (IOKAE). Here we present the first sedimentary province

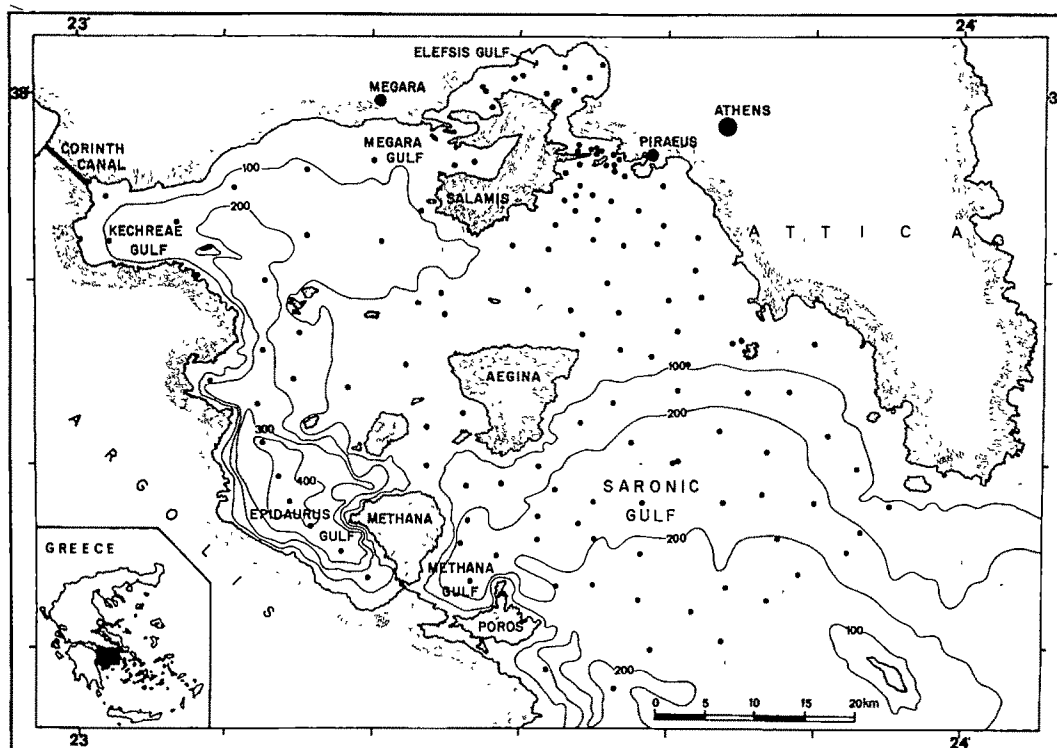


Fig. 1 Location, bathymetry and sample site map.

map of the gulf system, which expands current knowledge of the region and serves as a basis for further studies.

The only previous work of this nature done in the several gulfs comprising the Saronic system was contained in a biologic investigation by Vamvakas¹. He analysed 14 sediment samples from a limited area to determine the weight percentage of the coarse and fine fractions. Our observations confirm the results of his investigation concerning sediment characteristics and it is hoped that future research will delineate further the geological parameters and processes of the Saronic Gulf system.

The Saronic Gulf system as defined here incorporates the Saronic Gulf proper, as well as the Methana, Epidauros, Kechreae, Megara, and Elefsis gulfs (Fig. 1). It is bounded

on the west by the Argolis Peninsula of the Peloponnese, on the north and east by Attica, and opens to the south-east into the Myrtoon Sea. The seaward limit of the survey area is taken as a line from the south-eastern tip of Argolis to the southern extremity of Attica. The system has an area of 2,900 km² and, as compared with calculations by Hatzikakidis², comprises of the order of 10% of the continental shelf of Greece.

The depth of the eastern sector of the region ranges from <100 m in the Piraeus-Athens area, to between 100 and 200 m between Aegina and Attica, and is >200 m in a trough that extends from off the tip of Attica into the Methana Gulf. To the south, the depth shoals to less than 200 m. Elefsis Gulf is relatively shallow, less than 40 m

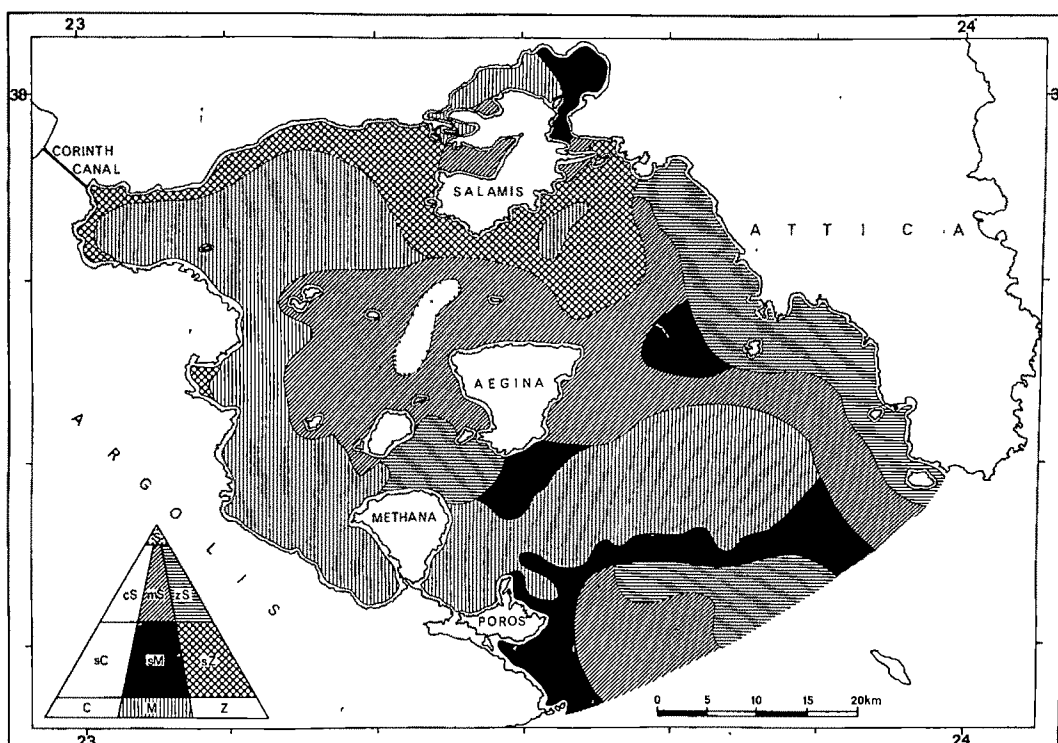


Fig. 2 Sediment distribution within the Saronic Gulf system. S, sandy; cS, clayey sand; mS, muddy sand; zS, silty sand; sC, sandy clay; sM, sandy mud; sZ, sandy silt; C, clay; M, mud; Z silt.

throughout. West of Salamis the Megara and Kechreai Gulfs exceed 100 m, and connect in the west with a north-south deep that reaches a depth of >400 m in the Epidaurus Gulf between Methana and Argolis. The Epidaurus Gulf deep and the Methana Gulf-South Saronic Gulf trough seem to bear a close correlation to pre-Pleistocene and Pleistocene normal faults, respectively, which transect the region. Further characteristics of the Saronic Gulf system have been described previously^{3,4}.

Source water to the Saronic Gulf is provided by a western boundary current in the Aegean Sea which flows past Attica into the gulf, and the annual circulation within the Saronic Gulf system has been investigated⁵. Wind generated waves are of limited fetch in all but the south-easterly direction, although seiches are occasionally generated by pressure disturbances passing over the area. Winds and barometric pressure changes contribute to a very slight Saronic Gulf tide with a range of up to 50–60 cm between the extreme high and low water marks. There are no major rivers draining into the basin and run-off from rivers and streams is insignificant except during periods of heavy, but short lived, rainfall which occur between late autumn and early spring.

The sampling programme was carried out during cruises aboard the Greek Hydrographic Service vessel *Anemos* and the Greek Navy minesweepers *Antiope*, *Niovi*, and *Phaedra*. Altogether, 131 bottom-samples were taken (Fig. 1) using Petersen, van Veen, and clamshell snapper grab samplers.

The colour of each bulk sample was determined, by comparison with the standard rock colour chart⁶. The portion of each sample subjected to particle size distribution analysis was first thoroughly mixed in a sodium hexametaphosphate (Calgon) solution (5.5 g l⁻¹), to achieve maximum dispersion. The sediment was then wet-sieved to collect the coarse, sand, fraction between 0.062 and 2 mm. The sand fraction was subsequently examined with the aid of a binocular microscope to ascertain its textural and compositional characteristics. For the purposes of this investigation an arbitrary classification was established whereby the sand fraction was called terrigenous if mineral and/or rock fragments comprised 40% or more, by estimated weight, of the fraction. No attempt was made to identify the organisms which constituted the organic component of the sand fraction (Vamvakas¹ has reported on this for the sector covered by his studies, and our 131 sand fraction samples are now undergoing species identification). Pipette analysis of the fine fraction was carried out, using 20-ml aliquots of the 4 ϕ and 8 ϕ classes at the appropriate times and depths, to determine the clay and silt content of the sample on a weight per cent basis. Based on these analyses of the sand-silt-clay ratio, each sample was then classified according to the system of Folk⁶.

It is assumed that basic factors affecting sediment distribution in this region are as follows. First, coarse terrigenous sediments, where present, are transported by wave action in coastal and shoal areas. The fine fractions settle in deep quiet waters or areas of low wave energy. Second, planktonic productivity increases toward the nutrient-rich waters in the north⁷. Benthic productivity, the main source of the biogenous coarse fraction, decreases with depth; a notable change taking place at the 200-m bathymetric contour¹. Third, yellow sediments predominate throughout the system. Where present, olive-coloured sediments denote reducing conditions caused either by poor circulation at depths or by wastes rich in organic matter, or contain ferromagnesian detritus. Finally the introduction of waste material into the system may inhibit benthic community growth and/or add fine particulate matter to the water column.

Figure 2 shows the sediment distribution within the Saronic Gulf system based on the locations of our 131 samples.

The investigation outlined here was conducted under the aegis of the Institute of Oceanographic and Fishing Research in Athens, with the cooperation of the Greek Navy and the Greek Hydrographic Service. M.L.S. was also supported by the Fulbright-Hays programme. We thank George Marinos, Ilias Mariolakas, Fotini Dousgou, and Joy Dabney for assistance.

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Stress on the bottom of an estuary

MANY estuaries are sufficiently deep relative to their maximum fetch that even in severe storms the bottom remains undisturbed by wind-generated waves. Yet observations of sediment transport and of changes in benthic animal populations indicate that agitation of the bottom of deep estuaries is not infrequent (refs 1–3, and D. C. Rhoads, unpublished). We report here an analysis of current meter records which shows that such bottom disturbances can arise because wind stress at the surface causes increased turbulence deep in the water column and, consequently, a more frequent occurrence of unusually high velocities near the bottom.

The records analysed were obtained at two locations along the central axis of Long Island Sound (LIS), an estuary 150 km long, 32 km wide at the widest point and with a mean depth of 17 m. The dominant water movement in LIS is the resonant co-oscillating tide⁴; tidal stream speeds reach 30 cm s⁻¹ at the locations studied. Currents were recorded with two types of meters: type I meters recorded instantaneous measurements of water velocity at time intervals which may be set from 1 to 30 min. Type II meters recorded the average speed and direction over successive 20-min intervals. All the meters were operated on taut moorings and were set 2 m above the bottom. The recorded velocities have been resolved into E–W and N–S components, approximately parallel and perpendicular to the axis of the estuary. Each time series of velocities is decomposed into a constant term, a group of periodic terms, and a fluctuating term. The periodic components have been removed from the velocity records by a least-squares regression of 18 sinusoids of tidal periods. The constant term is defined as the mean velocity over 3 or 12 h, depending on the time period chosen for calculating the variance or standard deviation. The residue remaining after removal of the mean and periodic parts is described as the fluctuating component, u' .

Wind data have been obtained from the US National Weather Service station at Stratford Point (41°10'N, 73°08'W), an exposed location on LIS. The data consist of 3-min averages of speed and direction, recorded every 3 h.

Figure 1 is a polar histogram of u' calculated from a record made by a type I current meter at Station A (41°09'N, 72°48'W,

depth=23 m) sampling the flow every 5 min for 4 d. The fluctuating velocities are isotropic. The square of the wind speed, proportional to the wind stress, and the standard deviation, σ_x , of u_x' over 3-h intervals are compared in Fig. 2a. The wind was from the north throughout the period of observation. Increases in wind stress result in increases in σ_x but the peaks of σ_x lag those of the wind stress by about 5 h. The time series of the E-W and N-S components of u' are quantitatively similar, as expected from the results in Fig. 1.

Two additional sets of data were obtained by sampling the current less frequently over a period of nearly one month at station B (41°08'N, 72°53'W, depth = 23 m). A type I current meter sampling every 30 min, and a type II meter were used. Wind stress data and σ calculated for 12-h periods are shown

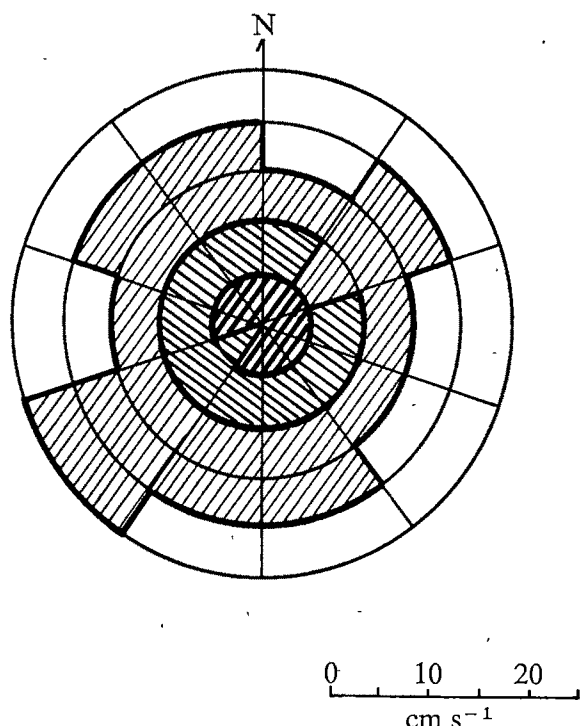


Fig. 1 Histogram of 951 observations of the fluctuating velocity component, u' , at station A. Darkest shading, probability of an observed velocity being in a box is $5\% < P \leq 7.5\%$; intermediate shading, $2.5\% < P \leq 5\%$; lightest shading, $0 < P \leq 2.5\%$. There is apparently no dependence of u' on direction.

in Fig. 2b and c. During calm weather σ drops to the range $1.5\text{--}2\text{ cm s}^{-1}$ identified as characteristic of the turbulence in the undisturbed tidal stream. Small increases in wind stress do not have much effect on σ but winter storms raise it by as much as a factor of 4 above the level associated with the tidal stream alone. The increase appears to be slightly smaller and the background somewhat higher for the type II meter, a consequence of the 20-min averaging performed in the meter itself. The magnitude of σ in the two data series in Fig. 2b and c is insensitive to wind direction. No correlation between u' and the phase of the tide is evident in the records.

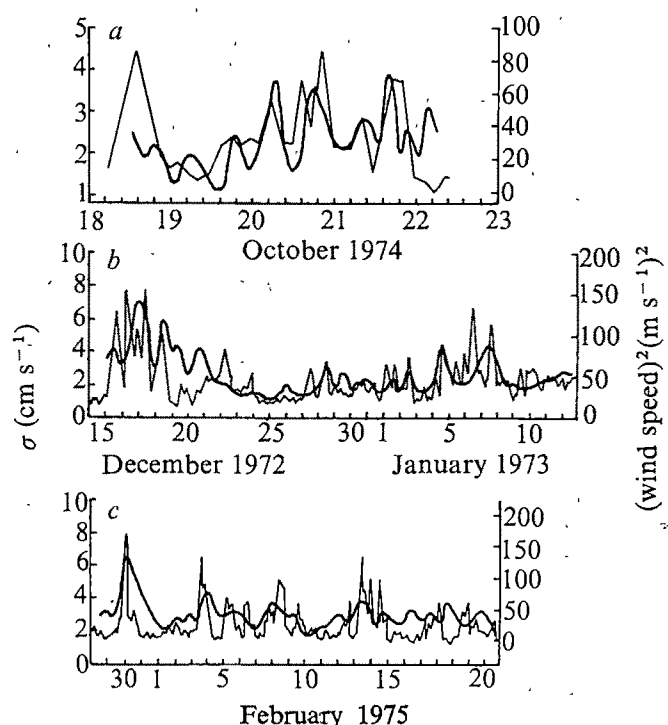
That the observed increase in u' does not result from disturbance of the meters by orbital motion of waves on the surface is shown by two lines of evidence: a water pressure recorder (wave gauge) was operated during the period February 19–March 27, 1975 on a reef at 41°00'N, 73°25'W, in water 14 m deep. Records of pressure fluctuations caused by waves on the surface were obtained when the wave period exceeded 4 s. (The recorder is insensitive to waves of shorter periods because of the large hydraulic attenuation.) There is a close correlation between the amplitudes of the pressure fluctuations and the factor v^2/f_m where v is the wind speed, f is the fetch for the

wind direction and f_m is the minimum possible fetch at the site. Throughout LIS large values of f/f_m occur only in the ENE direction. Pressure fluctuations at the recorder are detected only for easterly winds. The fetch in all other directions is too short to allow waves of long period to develop, even for very strong winds. All of the current meters operated in water deeper than that in which the wave recorder was placed so that, if disturbed by waves, the disturbance would occur only for easterly winds. In fact, σ_x and σ_y depend only on the wind speed; they are independent of wind direction. Examination of the magnitude of u' during the major storm of December 14–17, 1972 illustrates that point: strong winds from the ENE, which produced a storm surge with an amplitude of 1.2 m on December 15 were followed by WNW winds which produced a negative surge of 1 m. The magnitude of σ is significantly greater when the WNW winds are prevailing, at times when waves with long periods are absent.

A second line of evidence comes from the observation that the magnitude of u' is isotropic, as shown in Fig. 1, in the record obtained at location A. During the entire period covered by that record the wind direction was constant (from the north). Any direct contribution to u' from wave action should be greatest in the direction of wave advance, which is not observed.

The current meter records show that during periods of high wind stress the frequency of occurrence of high velocities near the bottom is increased. For example, $\sigma = 3.4\text{ cm s}^{-1}$ for the second 8.3 d of the record in Fig. 2b and the probability of occurrence of $|u'| > 10\text{ cm s}^{-1}$ is found from the distribution curve in Fig. 3 to be less than 1%. During the stormy period, the first 8.3 d of the record, $\sigma = 6.7\text{ cm s}^{-1}$ and the probability of encountering $|u'| > 10\text{ cm s}^{-1}$ increases to 12%. In an estuary the average kinetic energy resulting from storm-generated turbulence may be small compared to that generated by the tide, but the more frequent occurrence during storms of speeds greater than the maximum speed of the tidal stream, V , will have a large influence on processes characterised by a critical speed $v_c > V$ (processes such as the transport of the coarser fractions of the sediment). Very large speeds, such as

Fig. 2 Time series of σ (heavy lines) and the square of the wind speed (light lines). a, σ_x for a type I meter at station A with the wind speed advanced to lead the current by 5 h; b, σ_x for a type I meter at station B; c, σ_y for a type II meter at station B.



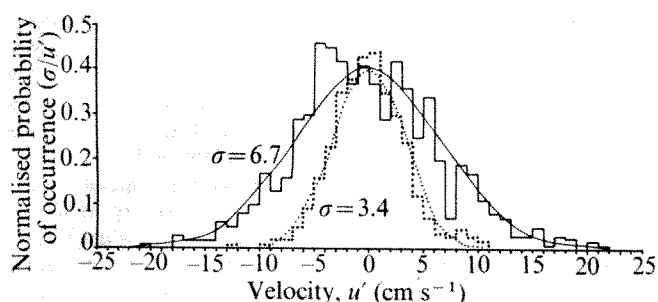


Fig. 3 Distributions of E-W fluctuating velocity components from the combined records of two current meters operating simultaneously 1.5 km apart at station B. Solid histogram data from an 8.3-d stormy period beginning on December 14, 1973; heavy dotted histogram, data for the subsequent, calmer 8.3 d; the solid and dotted curves show the normal distributions with the same mean and variance for each period. Each histogram contains 800 observations.

2V or 3V, are also much more likely to be encountered in storms, with a consequent disruption of benthic animal communities, even though the latter may be well below the depth directly affected by waves.

The data used here were obtained in studies for the New England Division, US Army Corps of Engineers and the United Illuminating Company of New Haven, Connecticut.

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First marine Triassic fauna from the Antarctic Peninsula

THE fauna reported here was obtained from the Legoupil Formation in the north-western part of the Antarctic Peninsula (Fig. 1), which has been assigned a Cretaceous age on the basis of radiometric data^{1,2}. Among the first collection of poorly preserved fossils from this formation were two bivalves tentatively referred to the Cretaceous genus *Platopsis*, an identification which substantiated the radiometric evidence. The study of a more recent collection, by members of the British Antarctic Survey, shows, however, that the bivalves have strong Triassic affinities and, since there is no reason to suspect that they are derived, indicates that the Legoupil Formation is of the same age.

It is also noteworthy that the sandstones and greywackes of the Legoupil Formation are lithologically and structurally unlike other known Cretaceous sequences in western Antarctica, more closely resembling rocks of the Trinity Peninsula 'Series'³⁻⁵ of possible late Palaeozoic age⁶⁻⁷. Both formations crop out in close proximity in the area discussed here, and the two have been confused in the field when the same outcrops have been assigned to both formations by different investigators^{2,3}. That reflects the similarity of the two rock groups and casts doubt on whether they are really distinct.

As yet, marine invertebrate fossils are unknown from the Trinity Peninsula 'Series' proper, and those from the Legoupil Formation are only known from a belt 100 m wide on the Duroch Islands (Fig. 1). The fauna is dominated by bivalves which occur mostly as moulds and are usually concentrated in

thin beds or decalcified coquinas. In spite of their unpromising state of preservation, several specimens still show sufficiently fine detail in the hinge region to allow reasonably confident identifications of following: *Myalinella* (?) sp., *Bakevelloides* aff. *hekiensis* (Kobayashi and Ichikawa), *Hoernesia* (?) sp., and *Neoschizodus* sp. nov.

Other fossils include a possible fragment of an inarticulate brachiopod, a gastropod, a serpulid, and some possible arthropod tracks.

Stratigraphically, the two most important species are *Bakevelloides* aff. *hekiensis* and *Neoschizodus* sp. nov. (Fig. 2). The best specimen of *Bakevelloides* (Fig. 2a) shows the ligament pits and pseudotaxodont dentition found in the type material⁸, but the dentition is better developed than in *B. hekiensis* which it otherwise resembles closely. Most species of *Bakevelloides* described seem to occur in the Upper Triassic of Asia. *Neoschizodus* sp. nov. (Fig. 2b) is the commonest element in the fauna. It shows typical myophoriid dentition and has a prominent myophorous buttress. It is closely related to the widely distributed *N. laevigatus* (Ziethen) of Lower to Middle Triassic age, but differs in having a radial riblet on the posterior area, and in possessing striations on some teeth. No specimens that

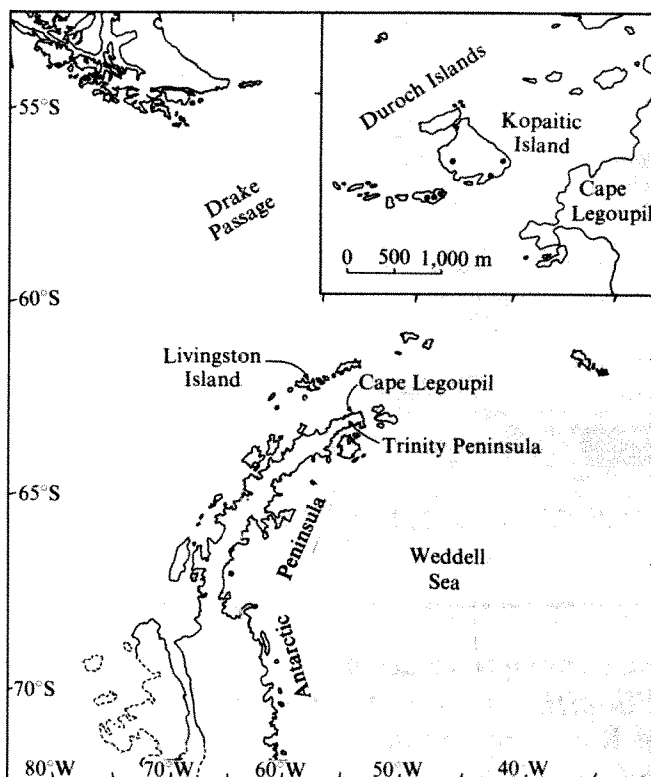


Fig. 1 Map of the Antarctic Peninsula showing the location of Cape Legoupil; inset, fossiliferous localities on the off-lying Duroch Islands (solid circles).

could be referred to *Platopsis* (?) sp. were identified among the collection. The only illustrated example of that species^{1,2} compares closely with the specimens here referred to *Neoschizodus*, and it is suggested that earlier ascriptions to *Platopsis* resulted from a misidentification.

This fauna provides the first evidence of marine Triassic rocks in the Antarctic Peninsula. The only previous fossil evidence for Triassic deposits in western Antarctica came from the discovery of poorly preserved plant fossils on Livingston Island¹⁰ but these were not collected *in situ*. Because of the close lithological and structural similarities between the Legoupil Formation and the Trinity Peninsula 'Series', it now seems possible that the upper age limit of the latter rock group could be younger than envisaged previously. Some tenuous supporting evidence has been cited from Livingston Island,

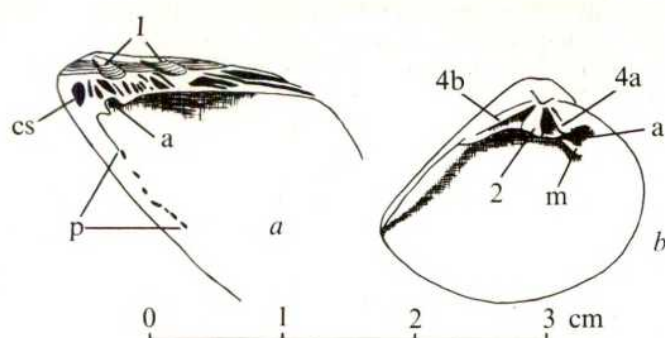


Fig. 2 Fossils from the Legoupil Formation: a, *Bakevellioidea* aff. *hekiensis* (Kobayashi and Ichikawa); b, *Neoschizodus* sp. nov.: a, anterior adductor muscle scar; cs, cardinal socket; l, ligament pits; m, myophorous buttress; p, pits along palial line.

where poorly preserved macroplant remains occur in the Miers Bluff Formation, previously correlated with the Trinity Peninsula 'Series' of the Antarctic Peninsula. That material suggests an age later than Carboniferous, perhaps even Mesozoic¹¹.

A detailed account of the fauna from the Legoupil Formation, and its implications, will be published elsewhere¹².

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New ramapithecines and *Pliopithecus* from the Lower Pliocene of Rudabánya in north-eastern Hungary

THE increasing interest of specialists in problems of human evolution has involved not only the final phases, which are well documented but not satisfactorily settled, but also the critical examination of earlier phases. For the latter, however, researches rely mainly on the Indian *Ramapithecus*¹ and the East African *Kenyapithecus*² maxillary fragments, while almost entirely neglecting the corresponding European materials. The reason for this may be that the European finds were scattered specimens, or insufficiently described and therefore very difficult to evaluate. In addition, they are described under too many, probably superfluous, names.

To fill this gap we here provide some preliminary data concerning recently found corresponding materials from Hungary. At the same time, however, we emphasise that for a more complete survey of the whole problem it will also be necessary to take the important fossil anthropoid materials from Spain³ and those found recently in the Near^{4,5} and Middle East.

There were two earlier finds in the Carpathian Basin, in East Alpine localities: Dévény-ujfalu (now Devínska Nova Vés, Czechoslovakia)^{6–9}, and St Stephan in Southern Austria¹⁰. A

very rich Lower Pliocene site, Rudabánya in north-eastern Hungary, recently yielded anthropoid material. The fossil-bearing strata belong to the lignitic Pannonian (Lower Pliocene) limnic sediments overlying the karstic depressions and valleys of the Lower–Middle Triassic range of the Rudabánya Mountains impregnated by a metasomatic iron ore body. The Pannonian strata contain, apart from the primate specimens, a very rich vertebrate fauna, a good mollusc fauna and a very rich flora (details of which will all be published elsewhere). The locality was rediscovered as an outstanding site of fossil hominoids by Mr G. Hernyák, chief geologist of the Iron Ore Works of Rudabánya, who has also collected the most important hominoid remains.

The flora indicates Mediterranean-subtropical climatic condition with traces of zonation ranging from aquatic through wet forest to grassland and higher mountain elements respectively. The basically Lower Pliocene character of the vertebrate fauna is proved by the presence of *Hipparion* in the fossil material. That it is a *Hipparion* fauna of archaic character is confirmed by the persistence of Miocene insectivore elements (*Trimylus*, *Miosorex*, *Dimylechinus*, *Plesiodimylus*, *Galerix*, *Lantanothierium*), the three hominoid forms, and especially the Miocene *Pliopithecus*, cricetids of Mio-Pliocene transitional type (*Cotimus*, *Democricetodon*), varied amphicyonids (*Amphicyon*, *Agnotherium*, *Rudacyon* n.g.), definitely Miocene carnivores (*Semigenetta*, *Sansanosmilus*), or archaic lagomorphs (*Amphilagrus*). At the same time, forms typical of younger *Hipparion* faunas are entirely missing, (for example, *Mesopithecus*, *Chalicomys*, *Neocricetodon* "Kowalskia") and especially all kinds of murids, hystricids and leporids, or ictitheriines, hyenas, true *Machairodus* forms, large agriotheriids (*Indarctos*, *Agriotherium*, *Agriarctos*), among ungulates primarily suids of 'Microstonyx' type, varied antelopes and all gazelles. All this evidence indicates that the fauna resembles that of Eppelsheim (Eppelsheimian), but certainly antedates it (Bodvaian) and thus should be dated as more or less synchronous with the invasion of *Hipparion* in Europe.

The anthropoid material is from 20 individuals (84 teeth altogether and 18 postcranial fragments) representing three different forms. One is a *Pliopithecus* species of great size

Fig. 1 *Rudapithecus hungaricus* Kretzoi, right mandibular corpus, Rud-1 (type). a, Lateral; b, occlusal view ($\times 1.8$).

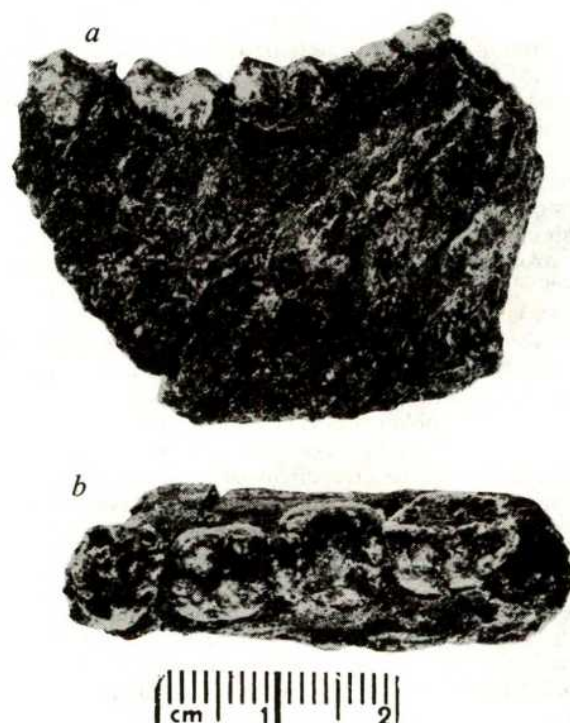


Table 1 Cranial and dental hominoid remains from Rudabánya

Specimen no.	Year collected	Specimen	Collected by*	Determination
Rud-1	1967	Mandibular ramus sin. (with P ₄ -M ₃) (Fig. 1)	G.H.	Rud. hung. (type)
Rud-2	1969	Damaged mandible (with C-M ₃ dext. and sin.)	G.H.	Rud. hung.
Rud-3	1971	M ₃ dext.	G.H.	
Rud-4	1971	C sup. sin. (♂)	G.H.	Pli. her.
Rud-5	1971	P ₃ dext. (germ)	G.H.	
Rud-6	1972	M ¹ sin.	I.V.	Rud. hung.
Rud-7	1972	Maxillary fragment (with P ⁴ -M ²) (Fig. 4)	G.H.	Bod. alt. (type)
Rud-8	1972	C sup. sin. (♀)	G.H.	Pli. her.
Rud-9	1972	Fragments of mand. dext. and sin. (with C, D ₄ , P ₄ -M ₂ dext. and M ₁ -M ₃ sin.) (Fig. 6)	G.H.	Pli. her. (type)
Rud-10	1973	P ³ sin.	G.H.	
Rud-11	1974	M ₂ dext. (germ)	G.H.	
Rud-12	1974	Left maxillary and palate (with I ¹ , C-M ¹) (Fig. 2)	J.H.	Rug. hung.
Rud-13	1974	M ² sin.	J.H.	Rud. hung.
Rud-14	1974	Mandibular fragments (with I ₁ -P ₄ , M ₁ -M ₂ dext. and I ₁ -C, P ₄ -M ₂ sin.) (Fig. 5)	G.H.	Bod. alt.
Rud-15	1974	Left maxillary (with I ¹ -M ²) and fragments of right one (with I ¹ -M ²)	I.P.	Rud. hung.
Rud-16	1974	M ₂ dext.	G.H.	Rud. hung.
Rud-17	1974	Corpus of left mandible (with C-M ₃) and fragments of left mandible (with I ₂ -M ₃) (Fig. 3)	K.B.	Rud. hung.
Rud-18	1974	M ² sin.	G.H.	Bod. alt.
Rud-19	1975	M ₃ sin.	G.H.	Rud. hung.
Rud-20	1975	C sup. dext. (♀)	G.H.	Pli. her.

Numbers are not Hungarian Geol. Survey inventory numbers.

*Names of collectors are: G.H., Gábor Hernyák; I.V., István Vörös; J.H., János Harnos; I.P., István Pálfalvy; K.B., Klára Beőreöndy.

(*Pliopithecus hernyáki* Kretzoi^{11,12}). The other two are pongo-hominids: *Rudapithecus hungaricus* Kretzoi 1967¹³⁻¹⁸ and *Bodvapathecus altipalatus* Kretzoi 1974^{11,12}. Brief information about the hominid materials is given in Tables 1 and 2.

The main characteristics of the new *Pliopithecus* species and the two pongo-hominids respectively are the following:

1. *Pliopithecus hernyáki* Kretzoi 1974^{11,12}. A species of the genus *Pliopithecus* s.l. of big size, with rounded pentagonal P₄, strong labial cingulum on the molars and well developed posterior fossa (triangular in M₃) with disto-lingual notch. The crest on the axial side of the protoconid (where it should meet the corresponding crest of the metaconid) is missing. Dimensions: D₄ 8.2-5.5 mm, P₄ 5.9-6.4 mm, M₁ 7.8-6.5 mm, M₂ 8.5-6.8 mm, M₃ 10.2-6.3 mm. Holotype: Rud-9 (Fig. 6)—The Rudabánya species seems to represent an independent superspecific taxon beside the described subgenera *Pliopithecus* s.str., *Epipliopithecus* and *Plesiopliopithecus*, for which the name *Anapithecus* n.sg. is suggested.

2. *Rudapithecus hungaricus* Kretzoi 1967¹³⁻¹⁸. A hominoid of slight build with a short face; high symphyseal, parasagittal, section of premaxillaries; wide incisive canal; flat palate; subparabolic dental arc; relatively small I¹; premolars subequal in size; low, brachyodont postcanines without cingula. Length of M₁-M₃: 29.5-31.0 mm. Holotype: Rud-1 (Fig. 1a, b), paratype: Rud-12 (Fig. 2a-d).

3. *Bodvapathecus altipalatus* Kretzoi 1974^{11,12}. A robust form of bigger size than *Rudapithecus*; high vaulted palate; relatively hypsodont post canines with marked cingula, roughly sculptured, thick enamel surface. The dimensions of M₁-M₃ are about 37-38 mm. Holotype: Rud-7 (Fig. 4a-c), paratype: Rud-14 (Fig. 5a, b).

There are some significant differences between the holotypes of *Ramapithecus brevirostris* and *Rudapithecus hungaricus*. *Ramapithecus* has a high, vaulted palate and the cross section

of the alveolar axis of the upper canine is longer and turned outward antero-laterally. (Former authors, like G. Lewis, failed to realise that the deformed alveolus of the canine gives the false impression of an abbreviated cross section.) *Ramapithecus* has a more antero-laterally situated I² so its dental arc is not subparabolic but more broadened in the frontal part. This means that *Ramapithecus brevirostris* (not identified with *Dryopithecus punjabicus* in my opinion), in spite of its younger geological age, is in some respects more ape-like, or more primitive than *Rudapithecus*, and represents a different line of hominid evolution.

Kenyapithecus with its flat palate, deep preorbital fossa, roughly enamelled and cingulated, higher-built postcanine dentition and somewhat bigger size, differs significantly from both *Rudapithecus* and *Ramapithecus*, in spite of the fact that most recent authors consider it identical to the Siwalik speci-

Fig. 2 *Rudapithecus hungaricus* Kretzoi, left upper jaw fragment, Rud-12. a, Lateral; b, occlusal; c, frontal; d, aboral view (×1).

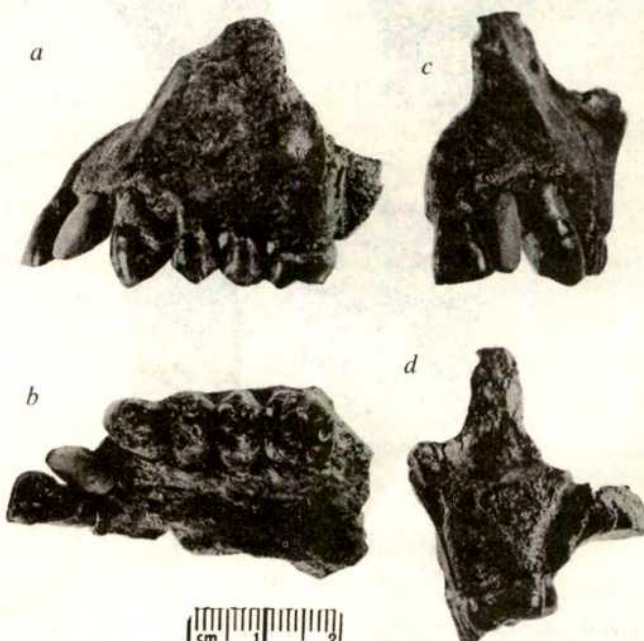


Table 2 Postcranial hominoid remains from Rudabánya

No.	Specimen	Determination
1	Distal fragment of left humerus	? <i>Rudapithecus</i>
2	Caput of the left femur	? <i>Rudapithecus</i>
3	Distal end of left femur	? <i>Rudapithecus</i>
4	Proximal end of left tibia (most probably of the same specimen)	? <i>Rudapithecus</i>
5	Left astragalus, without head	? <i>Rudapithecus</i>
6	Distal end of metapodial	
7	Distal part of phal. I	? <i>Rudapithecus</i>
8-9	Medial fragments of phal. I	? <i>Rudapithecus</i>
10-11	Distal parts of phal. II	? <i>Rudapithecus</i>
12	Distal two-thirds of phal. I	? <i>Bodvapathecus</i>
13	Distal part of phal. I	? <i>Bodvapathecus</i>
14	Phal. I, proximal end missing	<i>Pliopithecus</i>
15	Distal half of phal. I	<i>Pliopithecus</i>
16	Diaphysis of phal. I	<i>Pliopithecus</i>
17	Medial fragment of phal. I	<i>Pliopithecus</i>
18	Proximal end of phal. III	? <i>Pliopithecus</i>

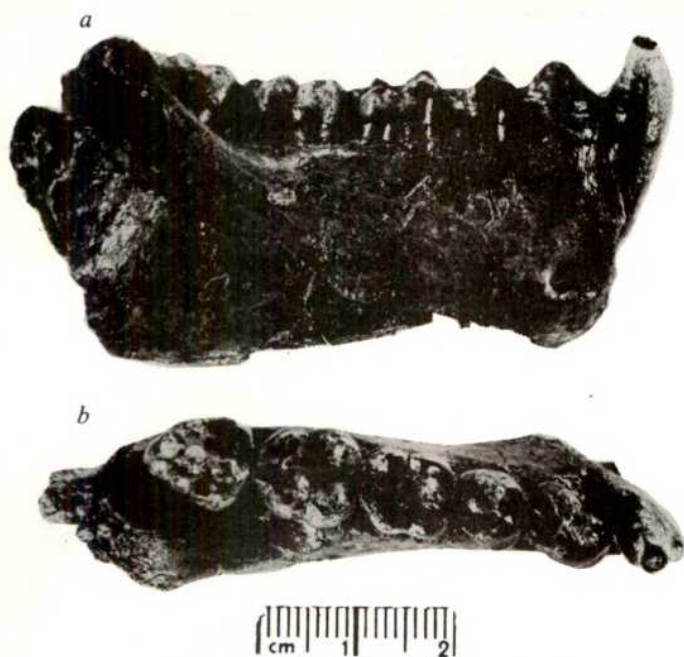


Fig. 3 *Rudapithecus hungaricus* Kretzoi, right mandibular corpus, Rud-17. a, Lateral; b, occlusal view ($\times 1.4$).

men. In addition to the differences between *Kenyapithecus* and *Ramapithecus*, *Rudapithecus* has, as distinct from *Ramapithecus*, an even flatter palate, in this respect resembling *Kenyapithecus*.

When comparing our material with anthropoid material considered to be such on the basis of their measurements, the situation is complicated by their poor preservation. We, therefore,

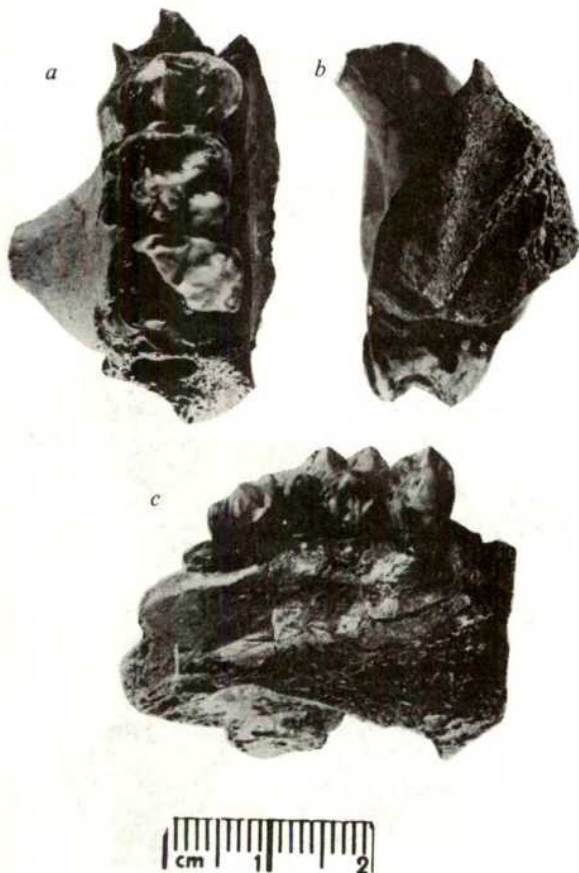


Fig. 4 *Bodvapihthecus altipalatus* Kretzoi right maxillary fragment, Rud-7 (type). a, Occlusal; b, aboral; c, lateral view ($\times 1.4$).

limit our comparison to the remark that the femur of the *Paidopithecus rhenanus*—reconstructed by mistake to about one inch longer than its real size—cannot actually differ greatly from *Rudapithecus*. It is not impossible, however, that the much-debated femur of *Paidopithecus rhenanus* is closer in size to the forms represented by the M_3 named *Neopithecus branconi* or the small molar referred to as '*Rhenopithecus*'. The scantiness of the *Hispanopithecus laietanus* and *Udabnopithecus garedziensis* materials make satisfactory comparison impossible. Apart from patterns common to all ramapithecines, or representing only the general trend of hominisation, there is no close relationship between *Bodvapihthecus* and the forms mentioned above, although the construction of its palate resembles *Ramapithecus* and its general dental trend *Kenyapithecus*. The two recently described finds from the Balkans (*Grecopithecus freybergi*⁴ and

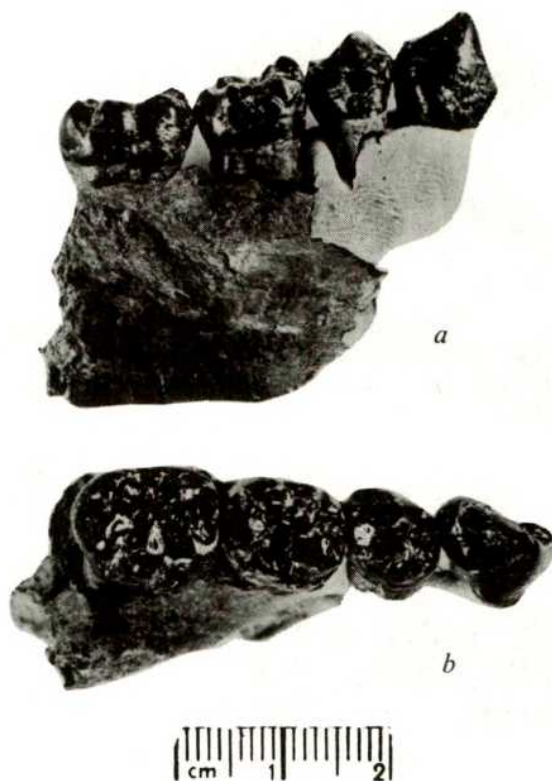


Fig. 5 *Bodvapihthecus altipalatus* Kretzoi, right mandibular fragment, Rud-14. a, Lateral; b occlusal view ($\times 1.5$).

*Dryopithecus macedoniensis*⁵) are larger and thus differ from both ramapithecines found in Rudabánya. Finally, *Dryopithecus keiyuanensis*, *Sivapithecus africanus*, *S. occidentalis* and *Rahonapithecus sabadellensis* ought to be mentioned and compared with our material as pongo-hominid forms tending towards hominisation. Because of their fragmentary representation or even lack of description, however, they are not really suitable for comparison. The same is also true of the remains described under the names of, for example, *Dryopithecus punjabicus*, and *Bramapithecus thorpei*. In these circumstances the most useful solution is to retain the identifications derived from well-preserved materials, suitable for further comparison, until the more correct names can be more precisely defined, hopefully, on the basis of further and richer material.

Although the taxonomic status of *Rudapithecus* is not influenced by its relationship to the forms representing the later phase of hominisation, it should be mentioned. The facial structure of *Rudapithecus* evinces a trend of hominisation which indicates a straight line of evolution through forms like *Pithec-anthropus modjokertensis* (restricted to the Sangiran IV maxillary) towards *Homo*. This seems to make it probable that australo-

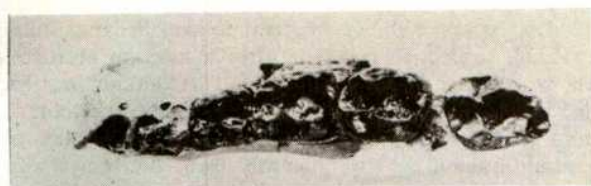


Fig. 6 *Pliopithecus hernyáki* Kretzoi left mandibular fragment with D₄-M₃, Rud-9 (type). Occlusal view ($\times 2$).

pithecines represent a close side branch with their independent development (decrease in size of front teeth, enlargement of molars, and so on), in many respects surpassing *Homo sapiens*, but not finally achieving the *Homo* level of evolution.

On the basis of the preliminary examination of the pongo-hominids of Rudabánya we can state the following conclusions: (1) Hominisation did not originate in certain isolated and more or less small tropical-subtropical gene pools, but was an evolutionary trend covering the whole Afro-Eurasian faunal radiation with its pongo-hominids. (2) Extant anthropoid apes, on the other hand, achieved their present non-human phase and trend of evolution as a result of a more or less extreme forest dwelling specialisation developed in certain isolated, tropical forest areas in Central Africa and south-east Asia. (3) Anthropoid evolution tending towards hominisation obviously developed on different levels, in numerous parallel, more or less 'human' branches which evolved so many parallel patterns during the process that it is not easy at present to find among them the single line which leads to the evolutionary development of *Homo sapiens*—and perhaps this is not even our primary concern at the moment, being more interested in collecting basic data. (4) The difficulties of identifying the previous, not satisfactorily defined, taxa make it necessary to use for the time being, the well-definable, later established taxonomic names.

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Tilt aftereffects with subjective contours

CELLS in the visual system respond well to spatial discontinuities in luminance (edges), but they are poorly activated by diffuse light¹. It has been proposed² that the selective response of edge-detectors to properties such as orientation and motion underlies form and pattern perception. In these terms, local discontinuity within the visual display is a necessary condition for perception of an edge. There are, however, anomalous contour effects³ (see examples in Fig. 1a and 1b) in which edges are clearly visible at sites where the visual stimulus is homogeneous. Such edges have been called subjective or cognitive contours on the supposition that cues to apparent depth within the display lead the observer to infer that he is seeing one plane located in front of, and interrupting, another plane^{4,5}. In these terms, "Since every plane must have an edge, the bounding contour is supplied by the perceptual system . . . a subjective contour is simply the edge of a subjective plane, and a

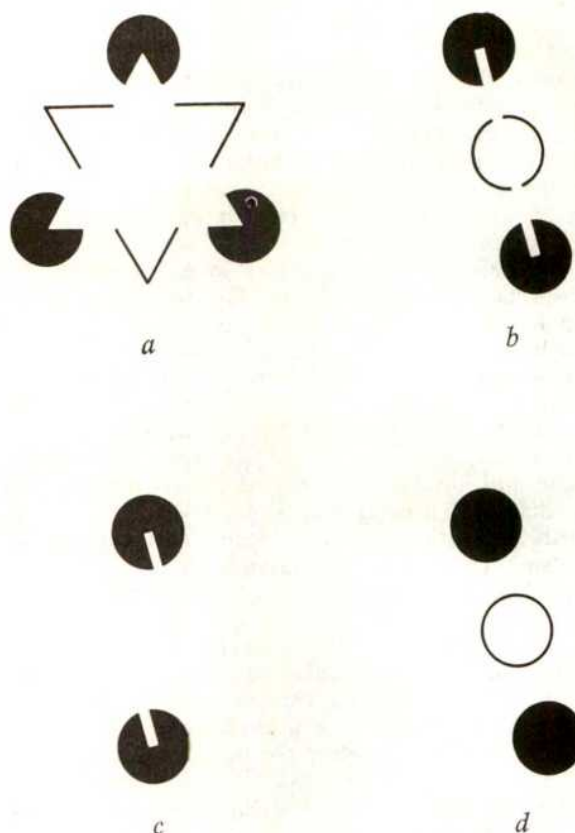


Fig. 1 Edges are visible within (a) and (b) in the absence of local luminance discontinuity. (b) was used as the subjective contour stimulus in the experiment. Test measures were also obtained following inspection of (c) and (d); these displays were used as control conditions in that they do not yield subjective contours but have some real contours in common with (b).

subjective plane is a surface which ought to be present on the basis of available depth cues, but is not except in the mind of the perceiver."

We report that the tilt aftereffect, in which a vertical line appears displaced clockwise after exposure to an anticlockwise tilted line, can be induced with subjective contours as well as with edges defined in terms of luminance discontinuity. In addition, exposure to a real contour results in a tilt aftereffect when the orientation of a subjective contour is subsequently judged, and vice versa. As the tilt aftereffect is generally attributed⁷ to adaptation of neural detectors which are tuned to contour orientation, these results raise the question of whether the perception of subjective contours has a basis at least in part in the activity of sensory feature detectors.

The experiment established the extent to which a test contour appeared displaced from vertical as a consequence of exposure to contours tilted 0°, 15°, 30°, 45° or 60° anticlockwise from vertical. This range of values was used because of evidence that the size of the tilt aftereffect depends on the difference in orientation between the inspection and test lines⁸. Aftereffects were measured for four conditions: inspection with real contour, test with real contour (R-R); inspect real contour, test subjective contour (R-S); inspect subjective contour, test real contour (S-R); inspect subjective contour, test subjective contour (S-S). The real contour, a white bar on a black ground, subtended either 4°36' \times 12' (inspection stimulus) or 3°4' \times 12' (test stimulus). The display shown in Fig. 1b was used to induce the subjective contour, which appeared in inspection and test conditions as a white bar subtending 4°36' by 12'. The top and bottom sections of this bar were

correlated with real contours, but the centre section subtending $3^{\circ}4'$ was visible in the absence of local luminance discontinuity.

On each trial a centrally located fixation point was shown for 2 s, then the inspection bar for 1 s, and finally the test bar for 100 ms. The observer's task was to report whether the test bar appeared tilted clockwise or anticlockwise from vertical; the use of other response categories was not permitted. The orientation of the test bar was varied over trials by a double-random staircase with 1° steps. For each of the four observers eight staircases were completed at the five inspection orientations for the four stimulus conditions. The order of testing conditions across observers was controlled by a Latin square, and orientation sequences were varied randomly between observers and conditions.

Figure 2 shows, for each stimulus condition, the mean orientation (displacement from true vertical) at which there was equal likelihood that the test bar appeared tilted clockwise and anticlockwise. The size of the aftereffect is given by the difference between values obtained when the inspection bar was vertical and those found after exposure to a tilted bar. An analysis of variance based on difference scores calculated for each observer showed that test judgments varied significantly as a function of the orientation of the inspection bar ($F(3,9)=32.30$, $P < 0.01$). The trends are consistent with other data⁸ on the orientational selectivity of the tilt aftereffect. The size of the aftereffect was independent of whether the inspection or test bars were formed by real or subjective contours ($F(3,9)=2.68$, $P > 0.05$), and the interaction between the orientation of the inspection bar and the type of display was also insignificant ($F(9,27)=0.86$, $P > 0.05$).

The possibility needs to be considered that judgments for condition S-S, S-R and R-S were under the control of the local luminance discontinuities within the display used to induce subjective contours instead of the spatial properties of the subjective contours themselves. It should be recognised that these two variables cannot be studied in isolation; the presence of real contour information is a necessary condition for the induction of subjective con-

tours. It is possible, however, to measure the apparent tilt of real and subjective test bars after inspection of patterns (such as those shown in Fig. 1c and d) that do not yield subjective contours although they have some real contours in common with Fig. 1b. Measures obtained in such control conditions differed significantly from the largest after-effects obtained for conditions S-S, S-R and R-S. The four subjects also judged the real and subjective test bars after adaptation to Fig. 1b without the broken circle at the centre, the broken circle alone, Fig. 1b with a complete circle at the centre and eight dots arranged as a bar. Mean displacements ranged from $+0.41^{\circ}$ to $+1.22^{\circ}$; all values were significantly less than the largest aftereffects shown in Fig. 2. These results can be treated as weak main effects rather than as control data in that the adaptation displays other than the eight-dot stimulus yielded slight subjective contours.

Our results indicate that the tilt aftereffect can be generated with subjective as well as with real contours. The orientational selectivity of the aftereffect is similar for the two types of stimulus. In addition, the apparent orientation of an edge that is correlated with luminance discontinuity can be shifted by previous exposure to a phenomenal edge at a location within the display that is uniform in luminance. In recent accounts neural edge-detectors have been implicated in the perception of real contours, whereas subjective contours have been treated as products of cognitive or inferential operations. Although data indicate^{9,10} that subjective contours cannot be explained within a simple sensory theory, our results suggest that it is premature to attribute the perception of real and subjective contours to fundamentally different processes.

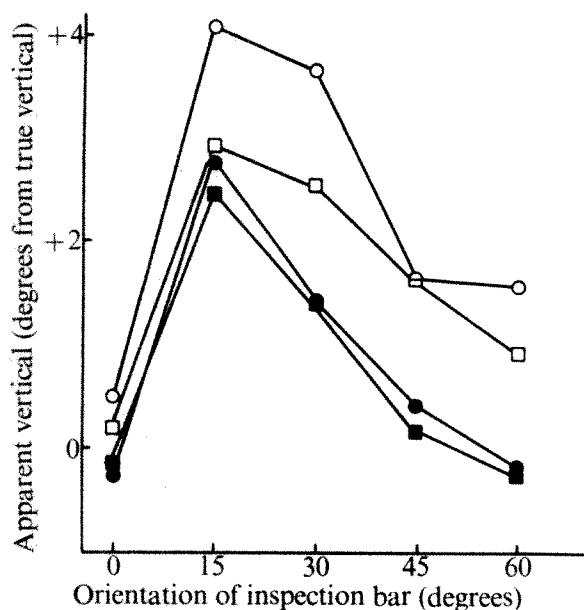
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Fig. 2 Mean location at which a test bar appeared vertical after exposure to an inspection bar tilted from 0 to 60° anticlockwise. Measures are expressed relative to true vertical, with clockwise displacements given negative scores and anticlockwise displacements positive scores. Values are shown separately for the four display conditions described in the text. ●, R-R; □, R-S; ■, S-R; ○, S-S.



Ambiguous cognitive contours

CONTOURS perceived in the absence of physical gradients of stimulation have been called cognitive contours¹, subjective contours^{2,3}, contours without gradients⁴, anomalous contours⁵ and quasi-perceptive margins⁶. The phenomenon is produced by placing inducing elements in special arrangements on a surface of uniform luminosity, as in Fig. 1. A whiter-than-white triangle can be seen overlying three black disks and a white background. The apparent brightness difference and the perceived contours extending between the disks are illusory. Gregory¹ discusses two paradigms, the cognitive and the physiological, which have been used to explain these contours. We introduce here two configurations which support the cognitive paradigm.

Figure 2 presents a subjective contour configuration which is perceptually ambiguous. Whereas Fig. 1 produces only one set of illusory contours (those corresponding to the 'sides' of the triangle), the 'ship's wheel' configuration can be perceptually organised in various ways, each resulting in an arrangement of illusory contours unique to that organisation. Three objects can be differentiated in the wheel-shaped structure of the figure; +, × and ○. These stratify in depth in a specific manner for any particular organisation of the figure. For example, the + can be seen overlying the ×, which in turn overlies the ○. The

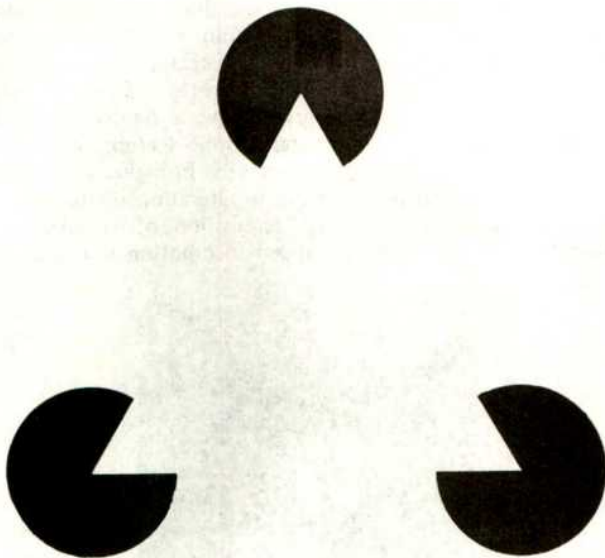
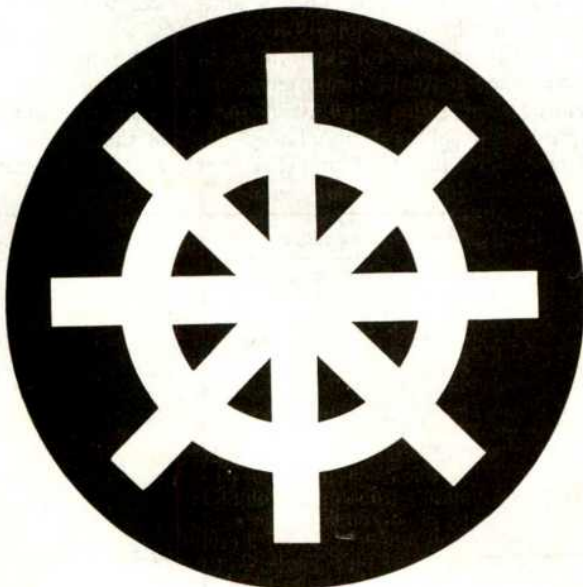


Fig. 1 A standard subjective contour configuration: a phenomenally complete triangle is perceived overlying three black disks.

bars of the + and the × are seen to pass over the ○, with illusory contours extending across it. The relative positions of these objects may spontaneously reverse, with the ○ now appearing on top. Curved illusory contours demarcating the phenomenal borders of the ○ are then seen extending across the bars of the + and ×. Alternatively, the + may be seen overlying the ○, and both of these overlying the ×. In this case, the straight subjective contours of the + are seen passing over the ○, and the curved subjective contours of the latter are seen passing over the ×. Clearly, there are as many possible arrangements of illusory contours as there are possible alternative organisations of Fig. 2, and the contours will be observed to 'shift' about the display during the transition from one organisation to the next.

Figure 3 presents another ambiguous subjective contour display. Illusory contours corresponding to the inside corners of a six-pointed 'star' may be seen extending between adjacent disks. Alternatively, an upright triangle can be seen overlying an iden-

Fig. 2 The ship's wheel configuration: the +, × and ○ alternate in apparent depth over time, thus causing the subjective contours corresponding to the phenomenal 'edges' of these objects to shift about the display. Subjective contours are not seen if the central white region is perceived as a completely solid object localised entirely in one plane (for example, a ship's wheel).



tical, but inverted, triangle. In this case, the subjective contours of the upright triangle are seen to proceed in straight lines, cutting across the 'corners' of the inverted triangle as they do so. If the relative positions of the triangles reverse, the subjective contours of the inverted triangle will now seem to cut across the corners of the upright triangle.

Corresponding brightness effects are associated with the alternative organisations of Figs 2 and 3. Objects which are seen as overlying other objects (or backgrounds) are perceived as somewhat brighter. For example, when the ○ is seen on top in Fig. 2 it seems somewhat brighter than the + and ×. Similarly, when the upright triangle is topmost in Fig. 3, it seems brighter than the inverted triangle which it overlies, and the latter, in turn, seems brighter than the background. When the perceptual organisation reverses the relative positions of the objects, the brightness relations reverse as well.

Since Figs 2 and 3 consist only of specially arranged inducing elements on white surfaces otherwise perfectly uniform in luminosity, the perceived contours and brightness effects are, of

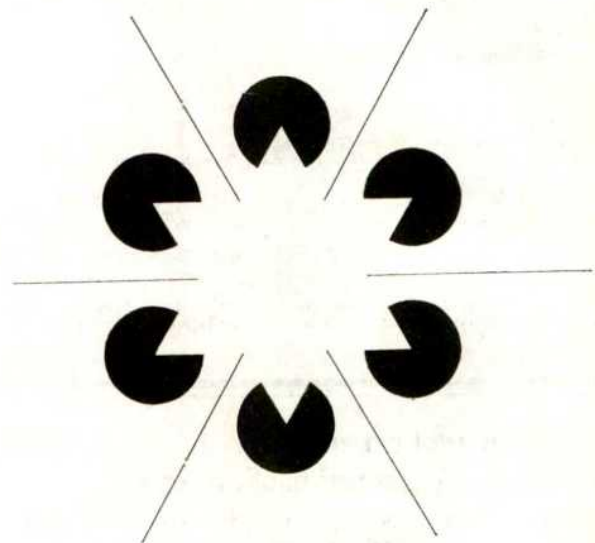


Fig. 3 The star configuration: either a six-pointed star or two superimposed triangles (with one inverted) may be seen. The perceived location of the illusory contours depends on the prevailing perceptual organisation.

course, illusory. The important point, however, is that different perceptual organisations of the same figure generate different illusory effects. Consequently, any theory which attempts to account for subjective contours must accommodate the fact that these phenomena are not totally stimulus-bound: that is, what is seen in Figs 2 or 3 cannot be predicted simply from a knowledge of the luminosity distribution of the physical array. Since physiologically oriented theories of subjective contours are often stimulus-bound in this sense⁷, they cannot explain our findings. The view that perception is a process of hypothesis formation in which 'object hypotheses' are selected by, but go beyond, sensory data⁸ is, however, consistent with these findings. If a configuration presents sensory data which can support multiple object hypotheses (Figs 2 and 3), then shifting illusory contours would be predictable as the result of alternative hypotheses being sequentially tested and applied to the ambiguous array.

There are two major limitations of the cognitive theory. First, the theory cannot predict which specific object hypothesis, of many possible, will be selected in a given instance. Second, the cognitive theory has not attempted to explain the brightness differences which are consistently observed in subjective contour configurations. Competing theories often start by explaining the brightness differences first, and then derive the contours from the apparent brightness gradients which result^{7,9}. Cogni-

tive theory holds that it is the formation of object hypotheses going beyond sensory data which gives rise to subjective contours, so it must be shown that the associated brightness effects arise concurrently with, or subsequent to, the formation of the contours, rather than casually preceding them. We would argue that the apparent brightness differences derive from a classic figure-ground phenomenon: that is, a figure will generally seem brighter or more intense than a background of equal reflectance value¹⁰. In so far as the central white area in Fig. 1 is conferred the status of an object (triangle), it will necessarily be seen as figure overlying a background. Consequently, its brightness will be enhanced and a whiter-than-white triangle will be perceived. This would also account for the reversals in apparent brightness seen in Figs 2 and 3 which arise when the perceptual organisation reverses the relative positions of objects in the display. As one object becomes 'figure' relative to another by overlying it, it will also take on a phenomenally brighter or more intense appearance¹¹.

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Development of cat visual cortex following rotation of one eye

EVER since the first description of the optical inversion of images within the vertebrate eye, philosophers and psychologists have pondered the question of how animals interpret the directions of objects in space. The physiological discovery of retinotopic 'maps' in the brain (such that the retina is represented in an orderly array in the tectal¹ and cortical² visual areas) seemed to provide an answer to the question. Neurones in these visual centres have receptive fields that are restricted to a particular region of the retina and could therefore preserve information concerning the positions of objects in the visual world.

Humans show considerable ability to compensate for prism-induced displacement³, partial rotation⁴ and even complete inversion⁵ of the retinal image. The physiological mechanisms underlying this capacity are not understood. On the other hand, amphibians never adjust their orienting behaviour to adapt to physical rotation of the eye⁶. Indeed, if the optic nerve

is severed at the time of rotation, the fibres regenerate and reinnervate the contralateral optic tectum, re-establishing the original relationship between retina and tectum and thus an inverted representation of the visual world⁷. These experiments suggest that lower vertebrates have a rigidly specified mapping system to the contralateral optic tectum, which is uninfluenced by visual experience, whereas in higher mammals visuomotor behaviour is responsive to alteration of the visual input. In fact in kittens initial acquisition of visually co-ordinated behaviour, such as guided locomotion and guided



Fig. 1 The kitten (K131) whose right eye was surgically intorted by 109°.

reaching, requires visual experience with feedback from movement⁸.

We have developed a technique for torsional rotation of the cat's eye (without sectioning the optic nerve, since it will not regenerate). The results of this procedure have provided information about the plasticity of visual localisation in cats, and about the genetically specified restrictions on the properties of cortical cells.

In three kittens less than 3 weeks old, one kitten that had been kept in total darkness until 5½ weeks old, and one normal adult, the insertions of all the extraocular muscles of the right eye were divided and the whole globe gently intorted, taking care not to stress the optic nerve or the blood supply to the eye. In each case the eye remained in its new position without suturing, and the conjunctiva healed very rapidly. Within a few days partial muscle reattachment occurred and limited eye movements returned.

All animals were then housed in a colony room (illuminated for 18 h each day), with both eyes open. Several weeks later the animals' vibrissae were cut and their visuomotor coordination was tested, using diffusing contact lenses to cover each eye in turn. Their behaviour was entirely normal through the non-rotated left eye and Table 1 summarises tests of their visual abilities through the rotated eye alone.

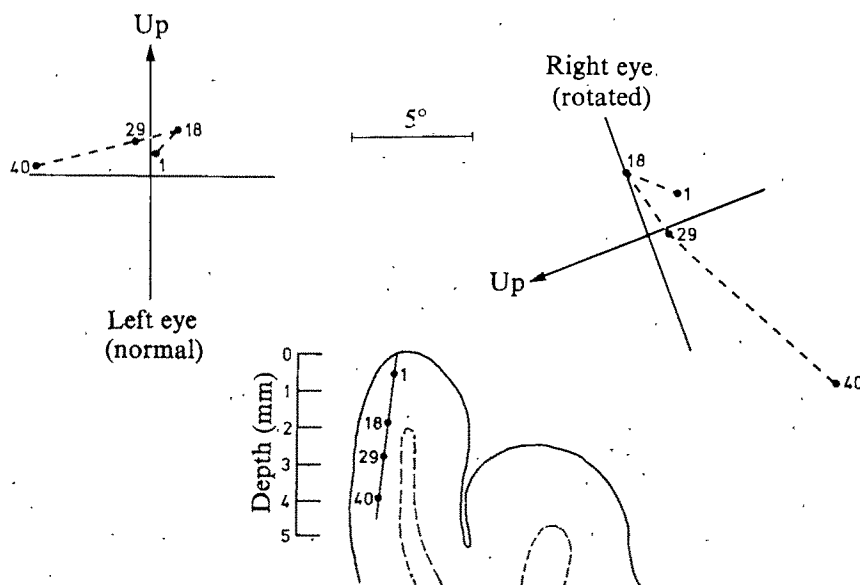
In all but one of these animals, with the normal eye occluded, head and eye movements could be elicited by presenting a small, high contrast object against a homogeneous background (visual following). Except in K108, the animal with the smallest amount of rotation (30°), however, the movements

Table 1 History of the animals and visual abilities using the rotated eye alone

Animal	Age at rotation	Age at recording	Angle of intorsion of right eye	Visual following	Visual behaviour using the rotated eye "Triggered" visual placing	Visually guided reaching	Guided locomotion
K108	11 d	20½ weeks	30°	+	+	+	—
K126	21 d	19 weeks	72°	+	+	—	—
K140	Adult	5 weeks later	106°	+	+	—	—
K131	16 d	17 weeks	109°	+	+	—	—
K137	38 d (previously dark-reared)	11 weeks	115°	—	+	—	—

+, Visual behaviour present; —, Visual behaviour absent.

Fig. 2 Preservation of approximate retinotopic projection in the kitten (K131) shown in Fig. 1, whose right eye had been intorted by 109° . The electrode penetration through the visual cortex of the right hemisphere is reconstructed in the lower half of the figure, and the locations of four binocularly driven cells are marked on the track. Above are diagrams of the positions of the receptive fields of these cells, as they appeared on the screen in front of the animal. The centre of each field is marked \bullet and they are joined, in series, with interrupted lines. The projections in space of the true retinal vertical and horizontal meridians are drawn as solid lines, intersecting at the projection of the area centralis. The upper visual field of each eye (the field of the inferior retina) is marked \rightarrow , labelled 'up', on the projection of the vertical meridian.



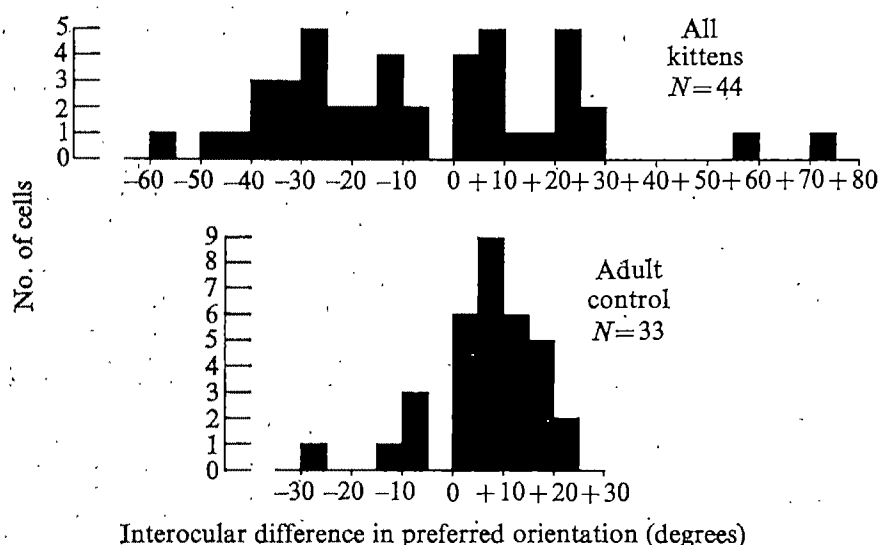
were rather jerky, unlike the smooth tracking seen in normal cats. Although the gross sequence was usually in the correct direction, the individual jerky movements may have contained inappropriate components, which were corrected by later movements. This unusual strategy of tracking deserves further study using more exact techniques for observing eye and head movement.

When lowered towards a broad horizontal surface, every cat extended its forelimbs downwards at an appropriate

of rotation, the responses were not very accurately directed in space. The lack of complete visuomotor compensation is, perhaps, not surprising in view of not only the rotation itself but also the relative immobility of the operated eye and the conflicting information from the other eye.

Behavioural compensation might be more complete with a longer interval between operation and testing, or if the normal eye were closed from the time of rotation. Both of these expectations seem to be confirmed by more recent experiments: two

Fig. 3 Analysis of the interocular similarity of preferred orientation for 44 binocular cells from kittens with eye rotation and 33 from the adult animal. The receptive field orientation was measured on retinal coordinates¹³ and, for each unit, the algebraic difference between the preferred orientations in the two eyes gave the value plotted on the abscissae of the histograms. Cells with identical orientation preference on the two retinæ have a value of zero. The distribution for the kittens, which had not had previous prolonged normal binocular vision, is significantly broader than that for the adult control ($P < 0.001$, F test, d.f. 43,32). The variance is not accounted for merely by pooling of data from several animals, since individual kittens showed very wide variability in the differences in preferred orientation.



distance from the surface ('triggered' visual placing). In normal cats such triggered extension is elicited only by approach to a horizontal surface: however, except for K108, movement of the limbs in the same direction sometimes also occurred if these animals were brought near a vertical surface.

K108 showed visually-guided reaching. When tested with an interrupted surface of the type introduced by Hein and Held⁹, this animal reached accurately for the prong, and avoided the gaps between prongs. In contrast, the animals with larger angles of rotation were equally likely to place the paw on a prong or in a gap. Finally, none of the animals managed consistently to avoid objects when moving through an obstacle course (guided locomotion). The results of eye rotation in the adult cat (K140) were not obviously different from those in kittens with comparable angles of rotation.

Thus the rotated eye was able to mediate behaviour to visual input, but, except for the animal with the smallest angle

of rotation, the responses were not very accurately directed in space. The lack of complete visuomotor compensation is, perhaps, not surprising in view of not only the rotation itself but also the relative immobility of the operated eye and the conflicting information from the other eye.

Behavioural compensation might be more complete with a longer interval between operation and testing, or if the normal eye were closed from the time of rotation. Both of these expectations seem to be confirmed by more recent experiments: two cats tested more than a year after 90° rotation are much more adept when using the rotated eye alone than any of the cats in the present study. A further animal with a 90° rotation that had the lids of the other eye sutured showed the best behavioural recovery of all, having quite reliable tracking movements, excellent performance on the obstacle course and accurate visually guided reaching.

vast majority of neurones were orientation selective, just as in the visual cortex of a normal cat¹¹. Only 5 out of 147 cells in the kittens and 0 out of 44 cells in the adult were unresponsive to our visual stimuli.

Photographs of the slit-shaped pupils taken before the preparation (see Fig. 1) and after paralysis enabled us not only to measure the surgically induced intorsion of the right eye (assuming that the two pupils should have been symmetrically orientated), but also to assess any further rotations of the eyes induced by paralysis. Thus we were able to relate the position and preferred orientation of each receptive field to the corrected horizontal and vertical meridians, on true retinal coordinates.

Most cells in our adult cat, as in normal animals¹¹, were excited by stimuli shown to either eye (35 out of 44 cells). Not surprisingly, however, the majority of visually responsive neurones in the younger animals (95 out of 142) were monocularly driven, just as in kittens with a surgically induced lateral deviation of the eyes¹².

The retinotopic map of the rotated right eye was virtually unchanged. Each region of the cortex represented approximately corresponding areas of the two retinæ, just as it had before rotation. Figure 2 shows the results for one animal (K131) whose right eye had been intorted by 109°. The histologically reconstructed microelectrode penetration is drawn below, and above it, just as they appeared on the screen, are the positions of the receptive fields of four binocularly driven neurones recorded at the points marked by filled circles on the reconstructed electrode track.

The projections on the screen of true horizontal and vertical retinal coordinates, intersecting at the area centralis, are shown for each eye. For this sequence of neurones the receptive fields in both eyes shifted from the vertical meridian, slightly into the field of the contralateral hemiretina, and then far out into the field of the ipsilateral hemiretina, typical of a penetration that starts in area 18 and moves deep into area 17. The other units recorded in this penetration, which were mainly monocular, had receptive fields that fitted this general progression. In every animal we observed this preservation of the normal retinotopic mapping from the rotated eye, and recently we have confirmed the finding in another kitten with a sample of receptive fields extending more than 30° into the peripheral retina.

Binocular cells in these kittens tended to have similar receptive field properties on the two retinæ in spite of the fact that there was no possibility of regular correlated binocular input after the rotation. In particular, we measured the preferred orientations on true retinal coordinates and Fig. 3 shows histograms of the differences between the two receptive fields for all binocular neurones that had clear orientation selectivity in both eyes (44 cells from the kittens, 33 from the adult). Cells from the normal adult with eye rotation had very closely matched preferred orientations on the two retinæ just as in normal cats¹³. Cortical cells in the kittens (who had not experienced a prolonged period of binocular vision before eye rotation) also, on average, had similar orientation preferences on the two retinæ. The range of differences (approximately $\pm 70^\circ$) was, however, much greater than in normal animals.

Thus there seems to be a definite innate restriction on the mapping from retina to visual cortex and, to some extent, on the interocular similarity of preferred orientations of cortical cells. Certainly the visually controlled behaviour displayed by cats with one eye rotated cannot be attributed to a reorganisation of the relationships between retina and visual cortex.

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We have recently learned that Yinon, Auerbach, Blank and Friesenhausen¹⁴ have also found binocularity to be reduced in the visual cortex of cats after rotation of one eye.

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Light-induced formation of dense-core vesicles in rod photoreceptors in retina of *Xenopus laevis*

SEVERAL attempts have been made to correlate changes in the fine structure of synaptic regions of vertebrate photoreceptor cells with dark or light adaption¹⁻³. Synaptic vesicles in both rods and cones were reported to be reduced in size after prolonged exposure to darkness^{1,2}. Later workers³⁻⁵, however, have not substantiated these claims. Mountford³ did not find any changes in vesicle populations in photoreceptors in response to changes in illumination; neither did Cragg⁴, although he did find that receptor terminals increased in width after prolonged periods in the dark, and decreased in width after only 3 min of exposure to daylight. Wagner⁵ showed in fish retina, that in animals kept under a constant day-night regime, the numbers of synaptic ribbons were reduced in fish fixed towards the end of the night period.

The upshot of this previous work is that there are no unequivocal descriptions of changes in vesicle structure which can be correlated with changes in illumination. We were therefore intrigued to find changes in the morphology of vesicles in the synaptic region of rods in the retina of *Xenopus*, brought about by exposure to continuous light. In the rods of toads kept in normal day-night conditions, or in constant darkness (up to 12 d), the synaptic vesicles surrounding the ribbons and in the neighbouring cytoplasm were all agranular (Fig. 1a). On the other hand, in animals kept under constant illumination (0.5 m distant from a

Table 1 This shows the mean % of dense-core vesicles in the synaptic vesicle population associated with synaptic ribbons relative to the period of exposure to continuous illumination

No. of days continuous light	Mean % of dense-core vesicles \pm s.e.	
4	24.55 \pm 2.38	$\left. \begin{array}{l} \ll 0.001 \\ 0.01 \end{array} \right\} P$
9	51.30 \pm 3.36	
12	13.88 \pm 3.29	

P is the probability between different treatments calculated by Student's *t* test. *P* > 0.05 not significant.

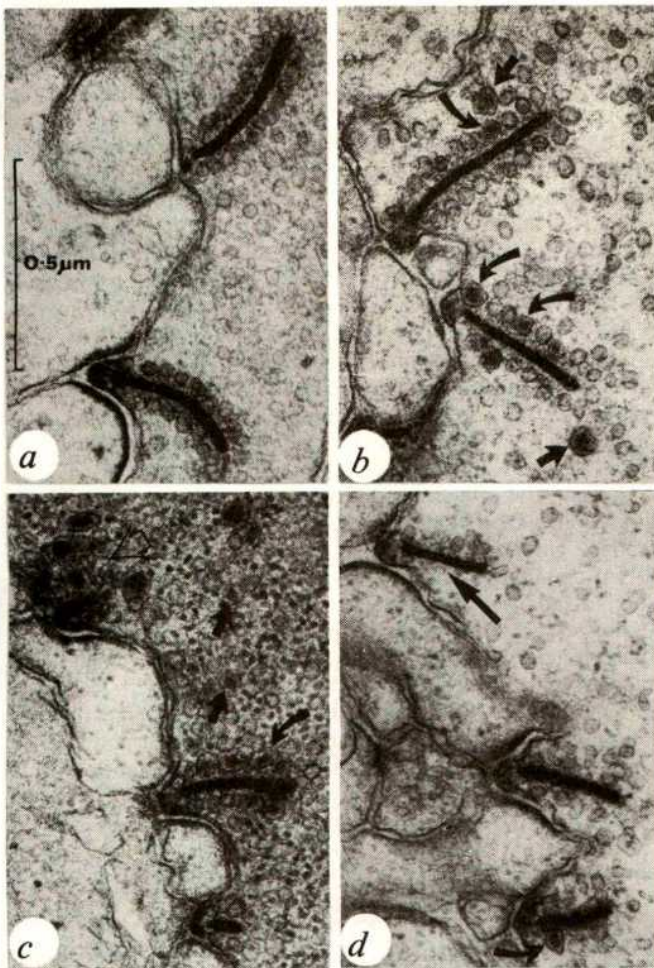


Fig. 1 Electron micrographs of rod pedicles of *Xenopus* retina from animals exposed to: *a*, day-night cycle; *b*, 4 d; *c*, 9 d; and *d*, 12 d continuous light. In (*a*) note that the three electron-opaque synaptic ribbons are surrounded by a cloud of exclusively agranular, synaptic vesicles. By contrast dense-core vesicles are present alongside the ribbons (curved arrows) in (*b*), (*c*) and (*d*) and in the cytoplasm (straight arrows) in (*b*) and (*c*). In (*c*), the open arrow indicates a clump of dense-core vesicles where the plane of section has just grazed a synaptic ribbon. In (*d*) note the absence of agranular vesicles from one side of a synaptic ribbon (straight arrow). Material prefixed in glutaraldehyde, postfixed in osmium tetroxide and embedded in epoxy resin. Scale line applies to all parts of the figure.

240 V, 40 W pearl, tungsten bulb; illuminance = 86.08 lx) after 4 d dense-core vesicles were found alongside the synaptic ribbons and in the surrounding cytoplasm (Fig. 1*b*, Table 1). By 9 d, on average, just over half the vesicles associated with the ribbons had dense cores (Table 1), although in some sections through synaptic ribbons all the vesicles were the dense-core variety (Fig. 1*c*). Dense-core vesicles were also more common in the rod cytoplasm (Fig. 1*c*). Another interesting feature in many rods was the presence of what appeared to be glycogen particles (Fig. 1*c*). After 12 d in the light, the number of dense-core vesicles was drastically reduced compared with that after 9 d (Table 1). Moreover, some agranular vesicles were lost from around the ribbons (Fig. 1*d*). Our results therefore suggest that constant illumination over several days induces the rods to produce dense-core vesicles, but interestingly enough, no similar structural changes in vesicles have been seen in the cones.

What physiological activities could underlie the appearance of these dense-core vesicles in rods? It is possible that because vertebrate photoreceptors are hyperpolarised by light⁶, that this will reduce their rate of neurotransmitter release⁵. In these conditions it might be expected that the

transmitter stores would increase. Such an increase in the transmitter store may account for the appearance of the dense-core vesicles and for their progressive increase in number during 9 d. The drop in their number by 12 d is puzzling, but it could be attributed to metabolic changes within the rods associated with a cessation or reduction of transmitter synthesis. This could also account for the reduced numbers of agranular vesicles associated with some of the synaptic ribbons (Fig. 1*d*).

The identity of the neurotransmitter(s) in rods and cones is not known⁷, although amino acids, catecholamines and acetylcholine are all possible candidates for synaptic transmitters in the retina⁸. Turtle photoreceptors can synthesise acetylcholine⁹, and there are high levels of taurine in the photoreceptor cells of the frog retina¹⁰. The occurrence of dense-core vesicles in the rods of *Xenopus* appear to be inconsistent with the presence of either of these two substances, since this type of storage vesicle is thought to be indicative of indoleamine and catecholamine stores in neurones⁸. Recent fluorescent and electron autoradiographic studies have demonstrated that although monoamines are present in the retina, they do not reside in the rods and cones⁸. It has been reported, however, that a fluorescent amine is associated with the nuclear layer of rods and cones¹¹, although the authors discounted that the fluorescence was caused by monoamines known to be neurotransmitters, that is dopamine, adrenaline and 5-hydroxytryptamine. The presence of an unidentified amine in the photoreceptors while possibly supporting our structural evidence of dense-core vesicles in the rods, conflicts with our failure to demonstrate dense-core vesicles associated with the synaptic ribbons of cones. It could be argued, however, that because cones are less sensitive to light than rods, the level of illumination in our experiments was insufficient to cause a buildup of dense-core vesicles in this type of photoreceptor. If this is not the case then it at least raises the possibility that rods and cones have different neurotransmitters.

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Tolerance of *Lolium* hybrids to quantitative variation in nuclear DNA

THE genus *Lolium* contains six species, all diploids with 14 chromosomes. The chromosomes of the inbreeding species are larger than those of the outbreeders and carry about 30% more DNA¹. Although the range of variation in nuclear DNA amount is not large, in comparison with other genera of flowering plants², *Lolium* is exceptional in that crosses between diploid species with widely different amounts of DNA produce F₁ hybrids which set viable seed on backcrossing or, alternatively, by intercrossing to produce F₂s. Segregation of nuclear DNA amount among hybrid derivatives by independent chromosome assortment and crossing over at meiosis in the

F₁ makes feasible a genetic assay of the effects of the differential interspecific DNA component. This report describes the segregation of nuclear DNA amounts among F₂ progenies (seedlings 5–7 d old) from F₂ hybrids between *L. temulentum* with high DNA and *L. rigidum* with low DNA content. F₁ seeds were obtained by allowing the F₁ hybrids to intercross in isolation from foreign pollen. From the DNA distribution in F₂ it is possible to draw conclusions about the influence of the differential DNA component on gamete and zygote viability. The results are given in Fig. 1.

As the result of recombination in F₁, DNA values range from approximately that of the low DNA parent to the high. Moreover, the distribution closely approaches and does not differ significantly from that of a normal curve. We conclude, in respect of the viability of gametes and of zygotes, toleration of a wide range of DNA amounts. Comparable quantitative DNA variation brought about by aneuploidy, that is by gain or loss of whole chromosomes, in *Lolium*⁴ as in most eukaryotes is the cause of severe genetic imbalance causing high mortality. We infer, therefore, that the 30% fraction of the DNA that distinguishes *L. temulentum* from *L. rigidum* is, genetically, of a substantially different character from that representative of whole chromosomes. Biochemical analyses in *Plethodon*⁵ have confirmed an earlier prediction⁶ that quantitative DNA

differences between related species involve mainly a particular and distinctive fraction of the nuclear DNA. The molecular distinctiveness finds a parallel in terms of genetic activity.

Growth and development of F₂ plants are normal in the sense that the variation in leaf production, shoot growth and flowering time lies within the range displayed by the parents. This is not to say, however, that the extra DNA in *L. temulentum* may be considered strictly inert. In this connection it will be observed that the mean nuclear DNA content of the F₂ progenies (23.6) is significantly lower ($P < 0.01$) than that of the mid-parent (F₁) value, 26.1. A possible explanation is that the development and viability of gametes or zygotes carrying mainly *L. temulentum* type chromosomes are at least partly dependent on the differential DNA fraction. Whether this interpretation is justified awaits additional evidence from more detailed assays.

The distribution of DNA amounts among the backcross progenies of *L. perenne* × *L. temulentum* given by Rees and Jones¹ leads to the same conclusions as those reported here. Above all, the evidence establishes, on a genetic rather than a biochemical basis, that the large scale variation in nuclear DNA amount among these eukaryote species involves a distinctive DNA component, distinguished by its relative inertness in comparison with that representative of whole chromosomes. In this respect there is an analogy with the DNA contributed by supernumerary B chromosomes⁵. Finally, since gametes and zygotes are tolerant of a considerable variation with respect to this nuclear DNA fraction it is the more surprising that the nuclear DNA amount within species of eukaryotes is so constant. The question arises as to whether the main restriction on quantitative change may, after all, lie more with the initiation of such change than with its consequences.

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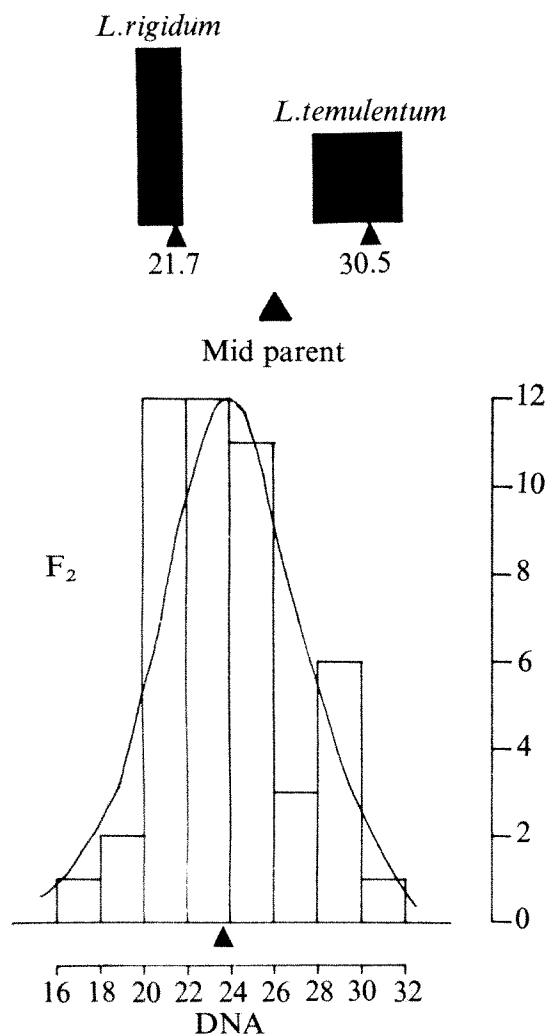
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Fig. 1 Nuclear DNA amounts (in arbitrary units) at G1 in *L. rigidum*, in *L. temulentum* and in 48 F₂ seedlings. Measurements were made on a microdensitometer (Vickers M85). Feulgen-stained root meristems were prepared by the method of McLeish and Sunderland³. Seedlings were handled in six batches of eight. A control (*L. temulentum*) was scored with each batch and adjustments were made to correct for minor fluctuations in readings between batches of slides. The theoretical normal curve is superimposed on the F₂ histogram.



Similarities and differences in latitudinal adaptation of two *Drosophila* sibling species

THE *melanogaster* subgroup in the *Drosophila* genus now includes six different species: four of them are found in tropical countries of the Ethiopian biogeographic region (*D. yakuba* Burla, *D. teissieri* Tsacas, *D. erecta* Tsacas and Lachaise, *D. mauritiana* Tsacas and David) whereas the two better known species, *D. melanogaster* and *D. simulans*, are widespread cosmopolitans. This geographical distribution is a strong argument for a tropical African origin of the subgroup¹ and it suggests that genetically only two species were sufficiently versatile for adaptation to northern and southern temperate climates.

Ecological observations, indicating that the two cosmopolitan species are linked to human activities², suggest that the species have been distributed by man during historical time; that they occupy similar ecological niches which are fairly constant all over the world; and that human transportation mixes the flies, resulting in a uniform genotypical composition throughout the range. Evidence is accumulating, however, that such suggestions may be incorrect and, consequently, that cosmopolitan species are indeed interesting for evolutionary studies³.

In *D. melanogaster*, linear latitudinal clines have been demonstrated for two quantitative polygenic traits (adult weight and female ovariole number) among temperate strains from Europe and North America, and tropical strains from equatorial Africa and America⁴. Preliminary studies suggested a similar phenomenon in *D. simulans*⁵. We have therefore undertaken a more extensive analysis. Results for 32 different strains of *D. simulans* (Fig. 1) are compared with the *D. melanogaster* data.

D. simulans is lighter and has fewer ovarioles than *D. melanogaster*; otherwise they are fairly similar. The fresh weight of females increases linearly with latitude and the slopes for the two species are otherwise not significantly different (the results for males (not shown) point to the same conclusion). For ovariole number, the increase for *D. simulans* falls off above 20° or below -20° latitude. From 0 to 20° the slope is significantly higher than from 20 to 45°. But these slopes are not significantly different from the single regression calculated for *D. melanogaster*.

As emphasised previously⁴ the adaptive significance of ovariole number and body weight must be explained by physiological traits such as reproductive capacity and resistance to environmental stress. Thus we decided to study another purely physiological trait, ethanol tolerance, the importance of which, in the ecology of the two species, has been discovered⁶. The variation of lethal concentration for adults as a function of

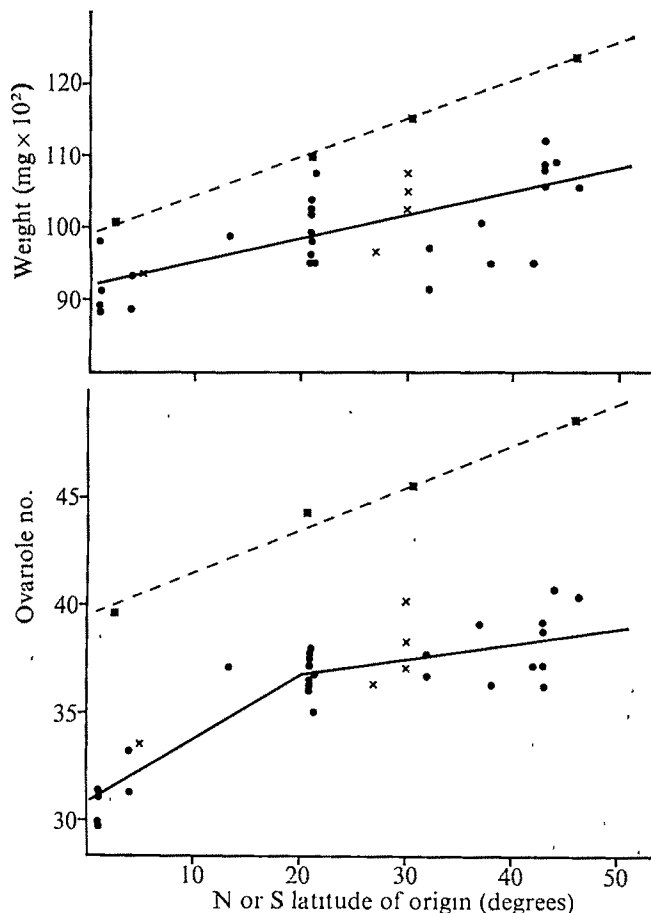


Fig. 1 Regression of female fresh weight and ovariole number on the latitude of origin of *D. melanogaster* and *D. simulans* strains. All measures were made on flies raised in standard laboratory conditions (25°C; killed yeast rearing medium; low larval density). For *melanogaster*, only the average points (■) of groups of strains are shown (see ref. 4). For *D. simulans*, each point corresponds to one strain: x, American strains; ●, European or African strains. Regression parameters for *D. simulans* (r , coefficient of correlation; b , slope; n , number of strains): Female weight: $r = 0.69$, $b = 0.315 \pm 0.059$, $n = 32$; ovariole number, below 20°: $r = 0.91$, $b = 0.293 \pm 0.033$, $n = 17$; 20–50°: $r = 0.51$, $b = 0.080 \pm 0.028$, $n = 24$. In *D. melanogaster*, regression parameters for 51 strains: female weight: $r = 0.93$, $b = 0.503 \pm 0.029$, ovariole number: $r = 0.77$, $b = 0.179 \pm 0.021$.

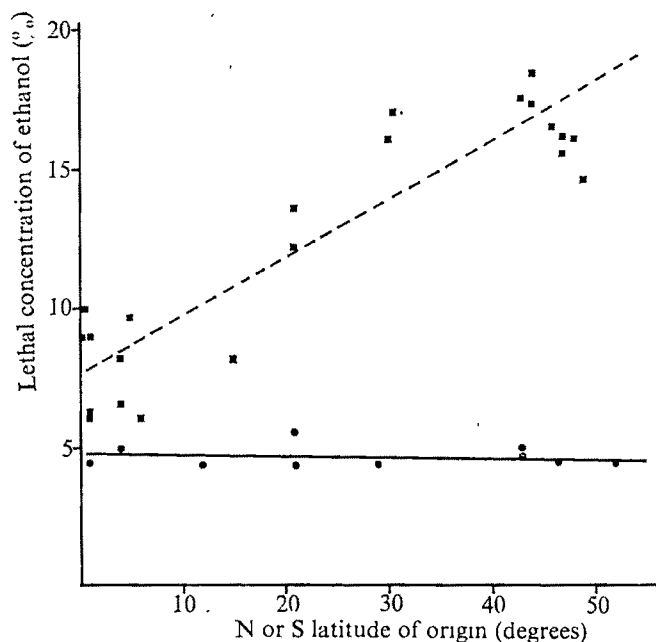


Fig. 2 Variation of ethanol tolerance in relation to the latitude of origin of the strains: ■, *D. melanogaster*, ●, *D. simulans*. Regression parameters (r , coefficient of correlation; b , slope; a , intercept, n , number of strains): *D. melanogaster*: $r = 0.90$, $b = 0.200 \pm 0.021$, $a = 7.69 \pm 0.63$, $n = 22$; *D. simulans*: $r = -0.23$, $b = -0.005 \pm 0.008$, $a = 4.79 \pm 0.25$, $n = 10$.

latitude of origin is shown in Fig. 2 (see ref. 7 for methods). In *D. melanogaster*, ethanol tolerance increases greatly with latitude; in *D. simulans*, however, the alcohol tolerance is lower and independent of latitude.

The higher ethanol resistance of *melanogaster* explains why it is the only species able to grow in highly alcoholic substrates such as fermented grapes in wine cellars^{6,8}. It seems probable that the geographical extension of *D. melanogaster* towards temperate countries occurred with a modification of its ecological niche, the species gradually becoming more linked to human activities such as artificial fruit fermentations. Much attention has been paid to the activity of alcohol dehydrogenase (Adh), which metabolises ethanol⁹. It now seems that alcohol tolerance is related to Adh activity (refs 8, 9, and B. Clarke, A. Elens and J. McDonald, personal communications). It is also known that, between the two widespread *Adh* alleles (slow and fast), fast usually shows a higher enzymatic activity¹⁰. Finally, a latitudinal cline (increase of fast frequency with latitude) has been demonstrated in the North American continent^{10,11}. All these data agree with the idea that the cline for alcohol tolerance described here corresponds, at a genetic level, to an increase of the fast allele frequency in temperate countries. Laboratory studies have demonstrated the selective value of the *Adh* alleles in experimental conditions^{9,12} and data from wild populations seem to demonstrate their selective values in nature.

The case of *D. simulans* is quite different, as the latitudinal adaptations seems to be independent of alcoholic food sources. This suggests that *D. simulans* may be less linked to (at least certain) human activities than *D. melanogaster*.

Latitudinal clines, on a shorter scale, have been demonstrated for biometrical polygenic traits in other (non-domestic) *Drosophila* species^{13,14}. The occurrence of similar data in two domestic species suggests that the natural selective mechanisms were the same—probably an adaptation to the average temperatures of the different regions.

An important problem is the time needed for the development of these genetic adaptations. Laboratory experiments indicate that less than 25 generations of artificial directional selection on equatorial strains are required to reach the higher weight, or higher ovariole number, of temperate strains. Moreover, experiments in population cages at different temperatures

have shown that biometrical characters of *D. pseudoobscura* can change significantly within a few years¹⁵. For *D. melanogaster* and *D. simulans*, the time needed for the genetic transformations induced by climatic changes is still a matter of debate. It may be that many thousands of generations were necessary for the fly populations to adapt from tropical to temperate conditions. Other species of the *melanogaster* subgroup and certain widespread domestic species such as *D. ananassae* have demonstrated an incapacity to extend their geographical distribution. We cannot, however, eliminate the commonly assumed possibility that the geographical extension of *D. melanogaster* and *D. simulans* is a recent event and that the colonisation of new regions was mediated by human transportations.

If this was indeed the case, a new question arises: why are so many tropical species, repeatedly introduced into temperate countries (for example, by escaping from laboratories) unable to found colonies? An answer may be found in the peculiarities of the genome of the cosmopolitan species. The study of allozymes, however, has failed to show systematic differences in level of polymorphism between cosmopolitan and other wild species¹⁶. Important differences exist in the chromosomal polymorphism of *D. melanogaster* and *D. simulans*: the former is highly polymorphic all over the world whereas the latter is monomorphic¹⁷. Such differences, however, seem to be of little importance for the adaptive capacities of the species.

Many problems thus remain in understanding the ecological genetics and the evolutionary capacities of the best known *Drosophila* species. A controversy has arisen over whether electrophoretic protein variants are "neutral" or "selective"¹⁸. Our data support a belief in the adaptive value of *Adh* alleles in nature. They also draw attention to the adaptive significance of biometrical polygenic systems which have often been neglected in current theories.

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Influence of selection pressures on enzyme polymorphisms in *Drosophila*

KIMURA^{1,2} suggested that the widespread enzyme polymorphisms revealed by electrophoresis in *Drosophila*, man and other outbreeding species are due to the selective neutrality, or near-neutrality, of most mutations. Others have argued that the observed genetic variation is adaptive, since electrophoretic variants may differ in their biochemical properties³, different classes of enzymes show conspicuously different levels of heterozygosity⁴⁻⁶, and natural selection in the laboratory may lead to detectable gene frequency changes at enzyme loci⁷.

Yamazaki and Maruyama^{8,9} concluded, from a survey of observed gene frequencies at 1,045 protein loci in a wide variety of species, that the data are compatible with the hypothesis of strict neutrality. A similar analysis¹⁰ of the data for three *Drosophila* species, however, has shown a significant excess of alleles with mean frequencies less than 0.1, by comparison with expectations based on the neutral infinite allele model of Crow and Kimura¹¹, presumably due to the accumulation of mutant alleles of minor deleterious effects¹².

In this report, a more extensive set of gene frequency data from electrophoretic surveys of species of *Drosophila* is analysed to assess the average magnitude of the selection pressures involved. The data are derived from 12 large scale studies of nine species of *Drosophila*¹³⁻²². The analysis involves only those enzymes for which at least three localities were sampled, and only those alleles reaching a frequency of 0.01 or more in at least one locality¹⁰.

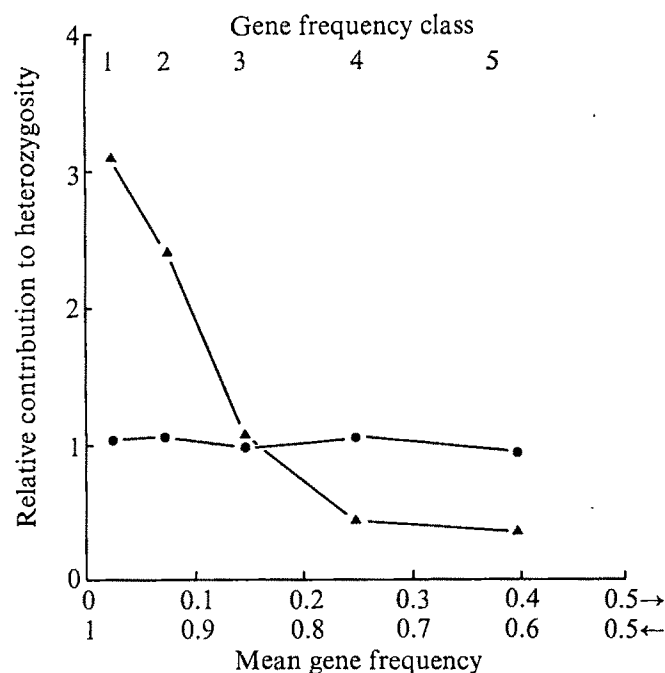


Fig. 1 The relative contribution of five gene frequency classes to heterozygosity for group I enzymes in *Drosophila* (▲), compared with expectations for the neutral charge class model (●) at equilibrium with $N\mu = 0.08$, determined by computer simulation.

Two types of genetic model have been used for comparison with the *Drosophila* data. The infinite allele model assumes that mutation to novel and individually distinguishable alleles occurs with frequency μ per generation. The charge class model, introduced by Ohta and Kimura²³, assumes that mutation occurs with frequency μ to novel alleles, of which a proportion α is not distinguishable electrophoretically from the parent allele, a proportion β gives rise to a protein differing by one unit of electric charge from the parental protein, and a proportion γ differs by two units of charge. Positive and negative changes in charge are assumed to be equally probable. Johnson²⁴ argued that the infinite allele and charge class models represent the two extremes of allelic non-identification when electrophoretic techniques are used.

The theoretical equilibrium gene frequency distribution in a random mating population of size N is known for the neutral infinite allele model²⁵, but not for the neutral charge class model. The equilibrium distribution for the charge class model has therefore been determined by computer simulation, using the values of $\alpha = 0.66$, $\beta = 0.32$, and $\gamma = 0.02$ calculated by Marshall and Brown²⁶ from the 'average protein' of King and Jukes²⁷, assuming that all mutations involve single random DNA base substitutions. The computer simulation was carried out with a population size of $N = 500$, allowing a period of

Table 1 Comparison of gene frequency distribution observed for group I enzymes in *Drosophila* and expected at equilibrium for genetic models involving slightly disadvantageous mutations ($Ns = 3$)

Genetic model	Relative contribution to heterozygosity of gene frequency class*					Mean heterozygosity
	1	2	3	4	5	
Infinite allele $N\mu = 0.14$	3.29 (± 0.11)	2.46 (± 0.06)	1.32 (± 0.04)	0.48 (± 0.04)	0.16 (± 0.05)	0.090 (± 0.002)
Charge class $N\mu = 0.40$	2.85 (± 0.14)	2.55 (± 0.09)	1.40 (± 0.04)	0.54 (± 0.03)	0.18 (± 0.04)	0.089 (± 0.003)
Group I enzymes	3.10 (± 0.45)	2.40 (± 0.38)	1.07 (± 0.22)	0.45 (± 0.12)	0.37 (± 0.14)	0.102 (± 0.012)

*Corresponding to mean gene frequencies falling in the intervals (1) 0.001–0.050 or 0.950–0.999; (2) 0.051–0.100 or 0.901–0.950; (3) 0.101–0.200 or 0.801–0.900; (4) 0.201–0.300 or 0.701–0.800; and (5) 0.301–0.700. s.e. of estimation is given in parentheses.

20N generations for the attainment of a stationary gene frequency distribution. Thereafter, the gene frequencies in every generation for all electrophoretically distinguishable variants were tabulated over a period of 200N generations. The standard errors associated with the frequency distribution and mean level of heterozygosity were estimated from the observed variance among independent computer runs.

Figure 1 shows the gene frequency distribution for group I enzymes, that is, those which take part in glycolysis, the citric acid cycle or hexose monophosphate shunt, or whose substrates are in one of these pathways²⁸, compared with that for neutral alleles using the charge class model with the same overall level of heterozygosity. The ordinate in each case is the relative contribution of each gene frequency class to the total frequency of heterozygotes^{8,10}. For each of the 12 *Drosophila* surveys the contribution of a gene frequency class to heterozygosity has been expressed as a proportion of the total frequency of heterozygotes, and the relative contributions averaged over species with weights proportional to the number of independent alleles involved¹⁰.

The departure of the *Drosophila* group I enzyme data from expectations based on the neutral charge class model is statistically highly significant: the same applies to the neutral infinite allele model²⁸. There is a marked excess of alleles with mean gene frequencies in the range 0.0–0.1 or 0.9–1.0, and a corresponding deficiency in the range 0.2–0.8 (Fig. 1).

To simulate the *Drosophila* group I enzyme data, it is only necessary to suppose that all mutations are very slightly disadvantageous. Table 1 shows the results of simulation of an infinite allele model and a charge class model in which each mutant has a selective disadvantage s as a heterozygote, compared with the homozygote for the allele from which it arose, with all genotypic fitness values being determined additively. This model has been analysed deterministically by King and Ohta³⁰. In the charge class model, relative electrophoretic mobility is determined by differences in electric charge alone, but reproductive fitness does not depend on the net charge of the protein molecule. A charge class may contain two or more alleles with different selective values. Table 1 shows that selective pressures corresponding to $Ns = 3$ are sufficient to account for the observed departure of the group I enzyme data from the neutral hypothesis.

The gene frequency distribution for group II enzymes, that is, those not involved in glucose metabolism, is shown in Fig. 2. The corresponding neutral charge class model with an equivalent mean level of heterozygosity is given for comparison. The excess of alleles at extreme frequencies is not as marked as for group I enzymes, but it is, nevertheless, statistically highly significant: the same is true of the neutral infinite allele model²⁸.

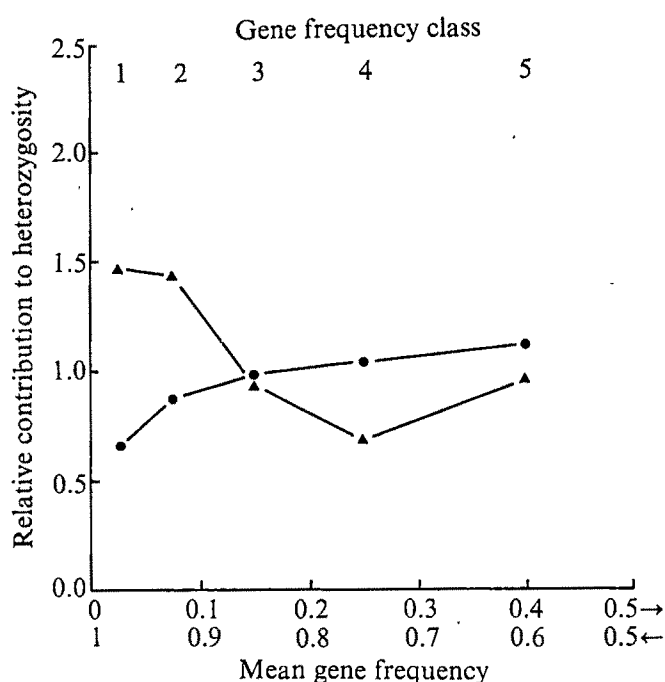
**Fig. 2** *Drosophila* group II enzyme loci (Δ) compared with expectations for the neutral charge class model (\bullet) with $N\mu = 0.25$.

Table 2 shows that the models of mutation to slightly deleterious alleles with additive genotypic fitness values give equilibrium gene frequency distributions similar to that of group II enzyme loci when $Ns = 1$. The fit is not completely satisfactory, however, the data departing significantly from the model in

Table 2 Comparison of the gene frequency distribution observed for group II enzymes in *Drosophila* and expected at equilibrium for genetic models involving slightly disadvantageous mutations ($Ns = 1$)

Genetic model	Relative contribution to heterozygosity of gene frequency class					Mean heterozygosity
	1	2	3	4	5	
Infinite allele $N\mu = 0.14$	1.45 (± 0.09)	1.45 (± 0.08)	1.24 (± 0.04)	0.93 (± 0.02)	0.69* (± 0.06)	0.208 (± 0.012)
Charge class $N\mu = 0.40$	1.15 (± 0.08)	1.36 (± 0.08)	1.26 (± 0.04)	0.98† (± 0.02)	0.75† (± 0.05)	0.190 (± 0.010)
Group II enzymes	1.47 (± 0.17)	1.44 (± 0.10)	0.94 (± 0.14)	0.68 (± 0.12)	0.96 (± 0.08)	0.226 (± 0.015)

*Significant departure from group II enzyme data at $P = 0.01$.

†Significant departure from group II enzyme data at $P = 0.05$. s.e. given in parentheses.

having an excess of allele frequencies in class 5, that is, in the range 0.3–0.7. Such an excess could be accounted for by two-allele polymorphisms at 5–10% of group II enzyme loci, maintained by balancing selection of sufficient intensity to keep gene frequencies in the range 0.3–0.7, with the remaining loci producing only deleterious mutants with N_s values of 2 or less²⁹. Among the group II enzymes, adenylate kinase, aldehyde oxidase and xanthine dehydrogenase contribute most to the excess of gene frequencies in the range 0.3–0.7.

It may therefore be concluded that the gene frequency data for group I and group II enzymes in *Drosophila* can be accounted for by mutation–selection balance at more than 95% of the loci concerned; and that the mean selection intensity influencing the deleterious mutant alleles at these loci is of an extremely low order, corresponding to N_s values in the range 1–3. Even if it were supposed that effective population size in species of *Drosophila* is as low as 1,000, the selective disadvantage of mutant heterozygotes implied by this analysis is of the order of 0.1–0.3%. Selection pressures of this magnitude are far too small to be detected experimentally³¹, and can only be revealed by extensive surveys of large numbers of enzyme loci.

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Evidence for a malaria mitogen in human malaria

HYPERGAMMAGLOBULINAEMIA is a prominent feature of human malaria. Serum levels of IgM and IgG rise rapidly in acute infections¹, and malaria has been implicated as an important cause of the raised levels of these immunoglobulins and the increased prevalence of rheumatoid factor and other autoantibodies found in healthy subjects in many parts of the tropics. Only a small proportion of the immunoglobulin produced in malaria can be shown to be specific antibody². The mechanisms underlying the production of large amounts of nonspecific immunoglobulin in malaria have not been established. Excessive immunoglobulin production could result from a breakdown in normal control mechanisms, perhaps as a consequence of the deletion of suppressor T cells. Alternatively, it could be due to the production during malarial

infection of a substance capable of stimulating lymphocytes nonspecifically³. Here we present evidence for the production of a mitogen in children with acute *Plasmodium falciparum* malaria.

Sera were obtained from 19 children with acute *P. falciparum* malaria at the time of presentation at hospital and from 16 healthy European adults taking regular malaria prophylactics. Blood for parasite culture was obtained from seven other children with acute *P. falciparum* malaria. None of these children had cerebral malaria or were clinically anaemic. Parasitaemia ranged from 1% to 15% parasitised erythrocytes with a mean of 7%. Blood for control cultures was obtained from six healthy Europeans who had never had malaria and who were taking regular malaria prophylactics. Heparinised blood, diluted 1.3 in RPMI 1640 medium, was passed through a column of CF 11 cellulose powder to remove white cells⁴. The diluted blood was washed through the column with RPMI 1640 and 5-ml samples collected. The first tube usually showed some haemolysis and was discarded. The next five or six tubes, containing only an occasional white cell, were collected and pooled. The red cells obtained were washed twice in fresh medium and a culture mixture was prepared containing 2 volumes of packed red cells, 2 volumes of inactivated foetal calf serum (FCS) or horse serum and 6 volumes of RPMI 1640. Culture was carried out in sterile conditions at 37 °C in 50-ml conical flasks loosely stoppered with cotton wool, in an atmosphere of 5% CO₂ in air.

After 24 h the culture was centrifuged and the supernatant retained. The remaining erythrocytes were washed twice in fresh medium, diluted 1.3 in RPMI 1640, layered over a Ficoll–Hypaque gradient and centrifuged at 400g for 30 min at 20 °C. This procedure removed remaining lymphocytes but resulted in the loss of some parasitised

Fig. 1 Mean stimulation ratio obtained on 3-d culture of lymphocytes from six immune Nigerians (●) and six non-immune Europeans (○) in the presence of different concentrations of supernatants obtained from culture of infected red cells. a, Supernatant 2; b, supernatant 3. Bars indicate the s.e.m.

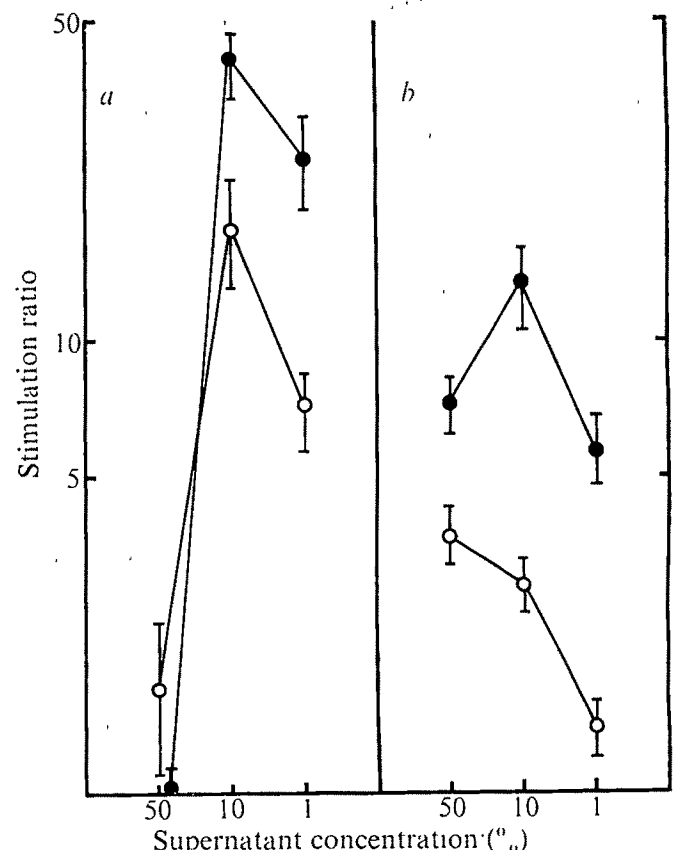


Table 1 Stimulation ratios

	50%	Supernatants 10%	1%	10%	Red cell lysates 1%	0.1%
Malaria cultures	2.0 (0.5-9.2)	4.2 (0.9-17.4)	2.0 (1.0-6.0)	2.4 (0.4-9.0)	1.9 (0.8-3.4)	1.5 (0.5-2.1)
Control cultures	0.9 (0.3-1.4)	0.9 (0.7-1.1)	1.0 (0.9-1.1)	1.0 (0.8-1.2)	1.2 (0.9-1.9)	0.7 (0.3-1.5)

Mean stimulation ratios were obtained on 3-d culture of lymphocytes from two non-immune subjects in the presence of different concentrations of supernatants and red cell lysates prepared from seven infected and six control erythrocyte cultures. Figures in parentheses indicate the range of stimulation observed.

erythrocytes and free malaria parasites. The sedimented erythrocytes were washed twice in medium and lysed with 0.87% ammonium chloride buffered with Tris-HCl buffer. After further washing the deposit was disrupted by sonication. The resulting supernatant, described here as a red cell lysate, was sterilised by Millipore filtration and stored at -40°C until tested. Protein concentrations of red cell lysate preparations were in the range of $1.3\text{--}5.5\text{ mg ml}^{-1}$. Blood from control subjects was treated in an identical way to blood from patients with malaria.

Sera, culture supernatants and red cell lysate preparations were tested for mitogenic activity on human peripheral blood lymphocytes and on mouse spleen cells. Lymphocytes were cultured in RPMI 1640 plus 10% FCS in microtitre trays at a concentration of 1×10^6 lymphocytes per ml for human peripheral blood cells and at a concentration of 3×10^6 lymphocytes per ml for mouse spleen cells. Eighteen hours before collection $1\text{ }\mu\text{Ci}$ of ^3H -thymidine was added to each well. Cells were collected on glass fibre disks with a Millipore manifold. Stimulation ratios have been calculated as the c.p.m. given by the test sample minus background divided by the c.p.m. given by the control sample minus background. Control cultures contained the same concentration of FCS or horse serum as the test samples. In each assay lymphocytes were tested against PHA at a final concentration of $50\text{ }\mu\text{g ml}^{-1}$.

Lymphocytes from a healthy European were cultured for 3 d in 10% serum obtained from 19 children with acute malaria and in 10% serum obtained from 16 controls. Three malaria sera, but none of the control sera, gave a stimulation ratio greater than 2 but other sera from the children with malaria were toxic, resulting in low cell viabilities and low stimulation ratios. Thus the mean stimulation ratio obtained on culture of lymphocytes in sera from children with acute malaria (1.0 ± 0.8) did not differ from the mean ratio obtained on culture in control sera (1.0 ± 0.5).

Supernatant and red cell lysate preparations obtained from culture of infected and control erythrocytes were tested for ability to stimulate lymphocytes obtained from two Europeans not immune to malaria (Table 1). Five out of seven supernatants and three out of seven red cell lysate preparations obtained from cultures of infected erythrocytes gave a stimulation ratio of 2 or more whereas none of six control supernatants or red cell lysate preparations gave stimulation of this degree. The mean stimulation ratio obtained with phytohaemagglutinin was 48.5 ± 19.6 .

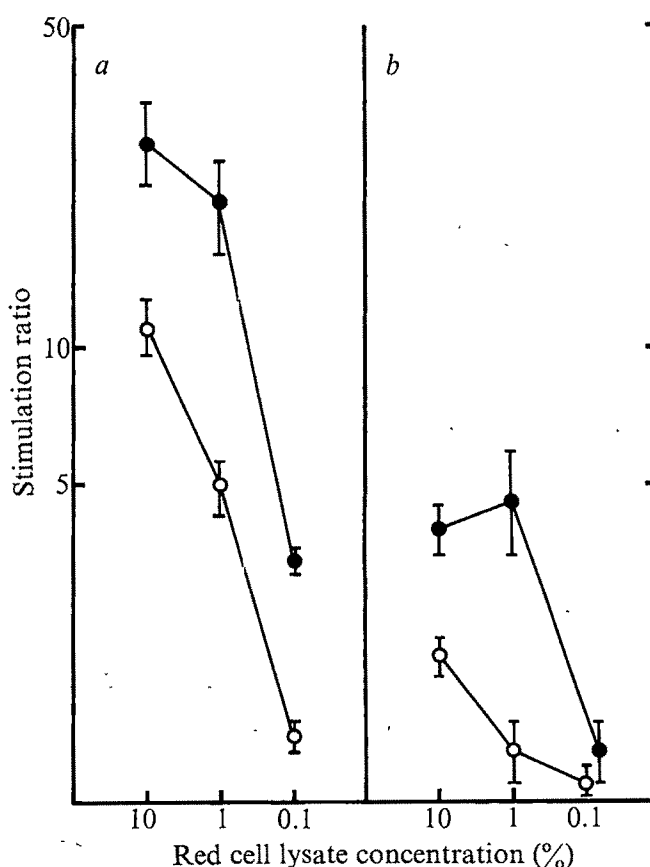
The two malaria supernatants and the two malaria red cell lysate preparations which gave the most marked stimulation in this initial experiment were tested for mitogenic activity on lymphocytes obtained from six adult Nigerians immune to malaria, on lymphocytes from six non-immune Europeans and on mouse spleen cells. Results for the two supernatants are shown in Fig. 1 and for the two red cell lysate preparations in Fig. 2. Although a wide range of responses was observed between different subjects each preparation gave a greater mean response in immune Nigerians than in non-immune Europeans. The different shapes of the dose-response curves obtained with supernatant 3 and red cell lysate 7 in immune and non-immune subjects also suggests increased sensitivity of lymphocytes

from immune Nigerians to these preparations. Lymphocytes from each subject responded to PHA with a mean stimulation ratio of 35.3 ± 19.7 for the European donors and a mean stimulation ratio of 12.3 ± 8.3 for the Nigerian donors. The low ratio obtained with lymphocytes from Nigerian donors reflects the high rate of isotope incorporation shown by their unstimulated cultures, a common finding in the local community. Mouse spleen cells showed a similar response to the two malaria supernatants and red cell lysate preparations as blood lymphocytes from non-immune Europeans.

A time-response curve was determined for supernatant 2 using lymphocytes from an immune and a non-immune donor. In each case maximal stimulation was observed around the third or fourth day of culture.

This study has shown that preparations obtained from short term culture of malaria-infected erythrocytes can stimulate lymphocytes from both immune and non-immune individuals. Leukocytes were removed from the culture preparations by an initial filtration through cellulose and by a subsequent Ficoll-Hypaque purification but the possibility that the stimulation observed in our experiments

Fig. 2 Mean stimulation ratios obtained on 3-d culture of lymphocytes from six immune Nigerians (●) and six non-immune Europeans (○) in the presence of different concentrations of red cell lysate preparations obtained from infected red cells. a, Lysate 2; b, lysate 7. Bars indicate the s.e.m.



was due to a mixed lymphocyte reaction, or even to a reaction to a red cell antigen, cannot be completely excluded. We believe this to be unlikely, however, for the following reasons. First, none of the control supernatants or red cell lysate preparations produced significant stimulation. Second, supernatants, presumably containing products released by parasite-lysed erythrocytes, produced more stimulation than cell-lysate preparations. The reverse situation would have been expected if a white cell or red cell antigen was involved. Finally, lymphocytes from those immune to malaria responded better than lymphocytes from non-immune subjects suggesting that the stimulatory factor was related to the presence of malarial products in the test preparations. It thus seems probable that the mitogenic factor demonstrated in our experiments was produced by malaria parasites or by parasitised red cells during the culture *in vitro*. Lymphocytes from malaria-immune Nigerians showed a greater response to the malaria culture preparations than lymphocytes from non-immunes, suggesting that these preparations contained both a malaria antigen capable of eliciting a specific cellular immune response by sensitised lymphocytes and a powerful nonspecific mitogen able to stimulate non-sensitised human peripheral blood lymphocytes and mouse spleen cells.

Our results thus agree with the finding that an antigen prepared by lysis of whole blood from monkeys infected with *P. falciparum* stimulated markedly lymphocytes from most subjects who had experienced malaria, and that at high antigen concentrations, lymphocytes from non-immune controls and even lymphocytes from cord blood responded⁶. These findings suggest this malaria preparation also contained both an antigen able to elicit a specific cell-mediated immune response to malaria and a nonspecific mitogen.

There is increasing evidence of the development of specific cell-mediated immunity during human malaria infection but it has yet to be established whether this has any protective role. It has been suggested that sensitised T lymphocytes function as helper cells, aiding the rapid production of antibody to newly arising antigenic variants⁶. Macrophage activation as a result of lymphokine production could also have a protective role by enhancing phagocytosis of parasitised erythrocytes.

The results of this study support the view that a mitogenic factor is produced during the course of human malaria infection but it has yet to be established whether it plays any part in the production of the hypergammaglobulinaemia characteristic of the infection. We are currently investigating whether it acts predominantly on B lymphocytes or T lymphocytes and whether it can stimulate immunoglobulin synthesis by lymphocytes *in vitro*.

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Lymphocyte activation *in vitro* to murine onco-foetal antigens

ONCO-FOETAL antigens (OFAs) are operationally defined as immunogenic cell surface components present on various types of malignant and foetal cells, but not present on adult cells^{1,2}.

As OFAs also seem to be shed from cell surfaces into blood and other body fluids, there is particular interest in the possible diagnostic value of detection of OFAs such as carcinoembryonic antigen^{1,2} and α foetoprotein³. In spite of many studies in this latter area, there is little information on either the basic function of OFA components or of their possible role in immune responses to tumours *in vivo*. Their frequent association with rapidly dividing cell populations suggests a role related to growth and differentiation rather than in the neoplastic process itself. Embryonic exposure of the normal individual to OFAs may induce immunological tolerance to these antigens, thus effectively negating any subsequent potential role of OFAs to act as tumour-associated transplantation antigens capable of eliciting an anti-tumour response *in vivo* in the host².

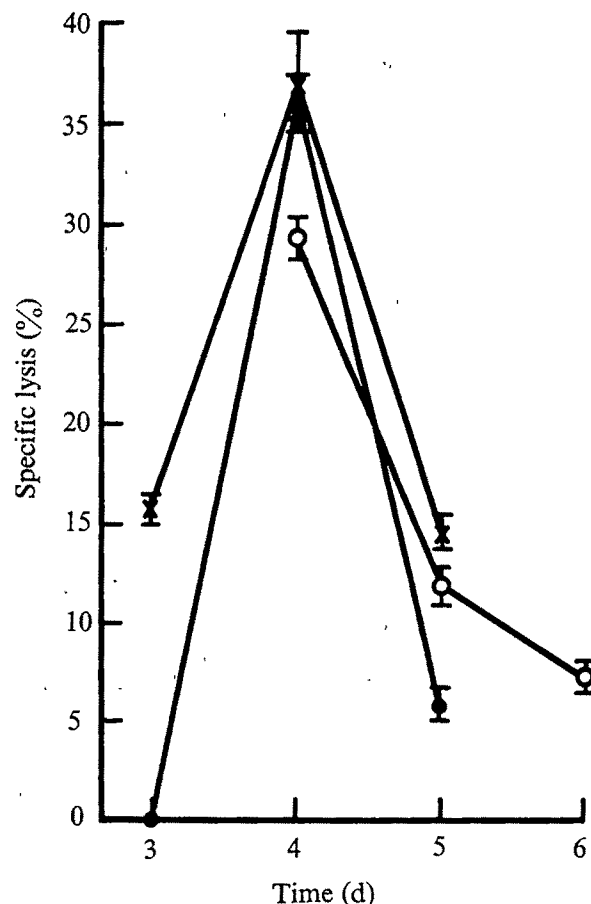


Fig. 1 Specific lysis of ⁵¹Cr-labelled target tumour cells by cytotoxic lymphocytes activated *in vitro* by syngeneic 14-d foetal liver cells, at a responder-stimulator ratio of 10:1. Cytotoxic lymphocytes were collected at indicated times (d) after initiation of culture, then held for 24 h in fresh medium before assay with ⁵¹Cr labelled MPC-11 cells (●, ○) or Wehi-164 cells (×). (CL-target ratio 100:1). Error bars are standard errors of triplicates.

For detailed investigations of this situation, animal models of OFAs are particularly appropriate, and various reports of OFAs on animal tumours have appeared^{2,4-6}. These include inhibition of chemical or viral carcinogenesis or suppression of tumour growth by foetal tissue immunisation, and in the reverse direction, inhibition of foetal cell proliferation by immunisation with tumour cells⁴⁻⁷. Analysis of the cellular mechanisms of immune responses to OFA in these situations has been limited, with one report demonstrating the presence of cytotoxic lymphocytes in the spleens of mice immunised with foetal cells, capable of lysing ⁵¹Cr-labelled tumour cells⁸. Analysis of the cellular components involved in anti-tumour responses has been aided by the development of totally *in vitro* systems for the induction and assay of anti-tumour immunity⁹⁻¹¹, making possible the control of variables which are difficult to evaluate *in vivo*. We now describe the *in vitro*

generation of cytotoxic lymphocytes by syngeneic foetal liver cells, and their ability to lyse syngeneic tumour cells.

The choice of foetal liver^{6,7} and the tumours used in this study was based on the results of investigations *in vivo*, demonstrating the ability of each tissue to provoke immune responses against the other (S.C., Wallis, and N.L.W., unpublished). The *in vitro* techniques used are described in detail elsewhere¹¹, and were briefly as follows. In all cases the microculture system of 4-ml compartments containing 15×10^6 responder cells was used. Irradiated (5,000 rad) BALB/c 14-d gestation foetal liver cells (stimulator cells) were cultured with 7-week-old male BALB/c spleen cells (responder cells) in Eagle's MEM containing non-essential amino acids, 5% foetal calf serum (FCS), and 10^{-4} M 2-mercaptoethanol at 37 °C in 10% CO₂ in air, using various ratios of stimulator-responder cells. At various times after initiation of culture, cells were collected and resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS for a further 24 h before assay. On the day of assay, tumour target cells were labelled with ⁵¹Cr in DMEM and mixed with the cultured lymphocytes at a cytotoxic lymphocyte (CL) to target (T) ratio of 100:1 and 50:1. After incubation at 37 °C for 4 h and 45 °C for 1 h, the amount of ⁵¹Cr released to the supernatant was measured. Background (BG) lysis was determined by measuring the amount of ⁵¹Cr released without CL, and the total amount of releasable ⁵¹Cr (maximal count) (MC) was determined by adding Zaponin (improved lysing agent for white blood cell counts). The percentage specific lysis is $[(\text{test count} - \text{BG}) / (\text{MC} - \text{BG})] \times 100$. The tumour lines used were a methylcholanthrene-induced BALB/c fibrosarcoma¹² WEHI-164, and a BALB/c plasmacytoma, MPC-11, (provided by Dr M. Scharff, Albert Einstein College of Medicine, New York). In several studies, inhibition of specific lysis was attempted by adding unlabelled foetal liver, adult spleen or tumour cells at various ratios to the ⁵¹Cr target tumour cells during the assay phase.

The generation of CL induced by syngeneic foetal liver cells and capable of lysing syngeneic tumour cells is demonstrated in Fig. 1. Culture of cells for 4 d before the preincubation and lysis assay resulted in optimal specific lysis of either tumour. In comparison the percentage specific lysis of tumour targets using BALB/c lymphocytes cultured with irradiated adult BALB/c spleen cells averaged $9 \pm 2\%$, regardless of assay day. Although there was some variation in the degree of lysis of the target cells from one experiment to another there was always at least a threefold increase of ⁵¹Cr release with BALB/c anti-foetal liver compared with anti-adult spleen. As other studies^{13,14} have inferred the *in vitro* generation of self-reactive CL, it is essential to make this comparison of adult with foetal stimulator cells in these studies. Our data on specific lysis with anti-self-reactive cells and the inhibition data below, do not suggest that the reactivity observed against foetal liver and tumour cells is of this auto-reactive nature.

Varying the ratio of responder to stimulator cells revealed optimal lysis at 10:1 with both tumour targets, whereas, in studies with specific anti-tumour immune responses, optimal ratios were 100:1 for MPC-11 (ref. 11), and 1,000:1 for WEHI-164 (R.B. and N.L.W., unpublished), again stressing the different type of antigenic system involved in the OFA study. Preliminary studies have also indicated that the gestational age of foetal liver effects the level of specific lysis generated, with a progressive decrease from 13 to 16 d of foetal age.

Evidence for specificity of cytotoxic lymphocyte activation comes from blocking experiments using non-labelled cells. Specific lysis of ⁵¹Cr target tumour cells by cytotoxic lymphocytes could be inhibited quantitatively by addition of foetal liver cells or Wehi-164 cells during the assay (Fig. 2), whereas there was little effect with adult spleen cells. In such experiments we consistently observed marked inhibition with Wehi-164 cells, but considerably less with MPC-11 cells, possibly indicating quantitative differences in the expression of OFA on different tumours. The degree of inhibition with foetal liver cells has been at least three times greater than that observed with adult

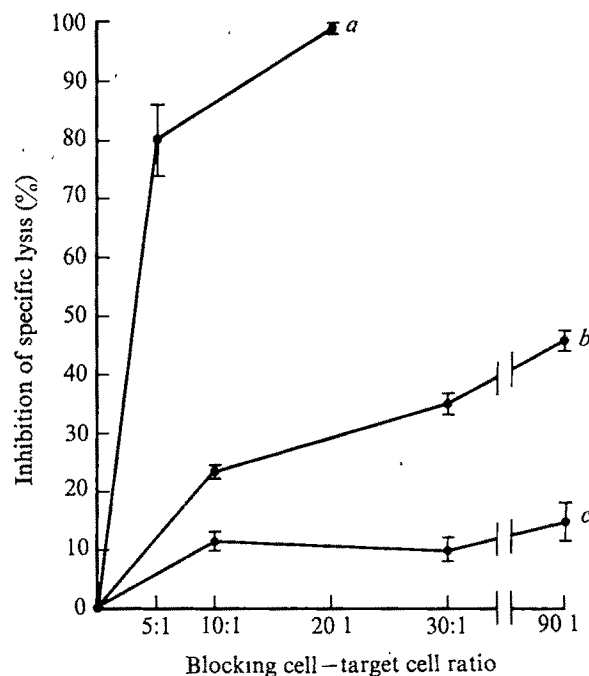


Fig. 2 Inhibition of ⁵¹Cr release from labelled Wehi-164 cells (CL-target ratio 50:1) by unlabelled Wehi-164 (a), 14-d foetal liver (b) and adult spleen cells (c) at various ratios of blocking cells to labelled target cells. The error bars represent standard errors of triplicate assays.

spleen cells, particularly at high blocking ratios. Whether the requirement for a relatively large number of foetal liver cells as either stimulators or blockers of CL reflects the expression of OFA on only subpopulations of foetal liver cells (for example, haematopoietic stem cells^{6,7}) or low amounts on most cells, remains to be elucidated. Preliminary studies have suggested the presence of OFA in foetal tissues other than liver. When degrees of blocking obtained with foetal or tumour cells are compared, it should be noted that the tumour cells used are of considerably larger volume.

The data presented here are consistent with the thesis that murine foetal cells have antigens in common with several murine tumour cells, which are not present on adult normal cells. The ability to generate *in vitro* cytotoxic lymphocytes to these antigens now permits further analysis of the tumour-host relationship involving OFA. As the two tumours used in this study do not apparently share *in vivo* tumour-associated transplantation antigens, and yet do share OFA, we are now determining whether the *in vitro* expression of CL represents T cell or B cell-mediated responses, perhaps released *in vitro* from a persistent *in vivo* suppression mechanism, as proposed for the *in vitro* generation of autoreactivity¹³.

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Altered serological and cellular reactivity to H-2 antigens after target cell infection with vaccinia virus

Mice generate cytotoxic T lymphocytes (CTL) which are able to lyse virus infected target cells *in vitro* after infection with lymphocytic choriomeningitis virus (LCMV) and pox-viruses¹⁻³. CTL kill syngeneic and semiallogenic infected cells but not allogenic infected targets. Target cell lysis in these systems seems to be restricted by H-2 antigens, especially by the K or D end of the major histocompatibility complex (MHC). In experiments where virus specific sensitised lymphocytes kill virus infected allogenic target cells⁴ the effector lymphocytes have not been characterised exactly. Recent investigations suggest that the active cell in this assay, at least in the measles infection, is a non-thymus derived cell (H. Kreth, personal communication). An H-2 restriction of cell mediated cytotoxicity (CMC) to trinitrophenol (TNP)-modified lymphocytes has also been described⁵. Zinkernagel and Doherty⁶ postulated that the CTL is directed against syngeneic H-2 antigens and viral antigens and they suggested an alteration of H-2 induced by the LCMV infection. Earlier⁷ we found a close topological relationship between H-2 antigens and the target antigen(s) responsible for CMC in the vaccinia system. Here we report experiments which were carried out to prove alteration of H-2 after infection of L-929 fibroblasts with vaccinia virus.

Alteration of H-2 antigens was studied by immunofluorescence. L-929 cells after 1 h infection with 10 TCID₅₀ per cell vaccinia virus strain WR and 19 h incubation in serum-free medium, as well as normal L-929 cells were stained by indirect immunofluorescence. L cells (5 × 10⁶) were incubated in 100λ H-2^k (charge D 3b, raised in recipient-donor strains (C3H-H2^k × 129) anti-C3H; genotypes (K^dD^k × K^bD^b) anti-K^dD^k. This serum is cytotoxic for H-2 specificities 11, 23, 25, 52 and haemagglutinating for specificity 3, (ref. 8) for 30 min, 37 °C and 4 °C and after washing 3 times in PBS incubated in 100λ FITC-labelled rabbit anti-mouse IgG in the same conditions. After the cells were washed three times, viability was tested (> 90%) and slides were prepared without previous fixation of cells. All of the normal fibroblasts had a dense and regular fluorescence, whereas about 25% of the infected cells lacked H-2 fluorescence. The remaining 75% cells had an irregular and more coarse-grained staining.

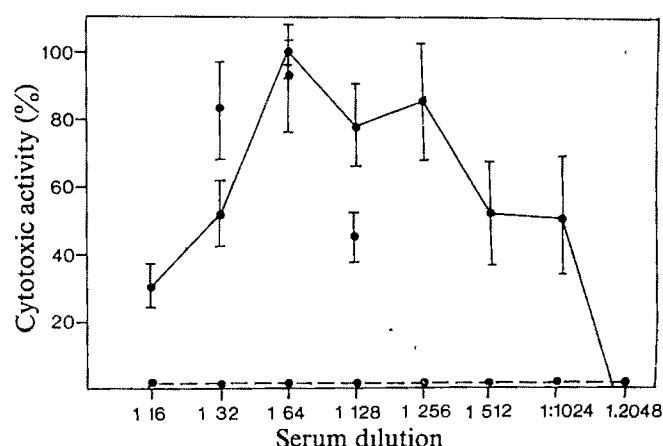


Fig. 1 H-2^k alloantiserum absorption with normal and vaccinia infected L-929 cells. ●—●, cytotoxic activity of anti H-2^k serum (⁵¹Cr release) against C3H spleen lymphocytes. ○—○, cytotoxic activity of anti H-2^k serum after 30 min absorption at 37 °C with 1 × 10⁸ vaccinia virus infected L cells (20 h infected 10 TCID₅₀ per cell vaccinia strain WR). ●—●, cytotoxic activity of anti H-2^k serum after absorption with 1 × 10⁸ normal L-929 cells. Serum dilutions were performed in Eagle's MEM. Each point represents N = 4 values ± s.d. Differences are statistically significant (P < 0.001). Maximal ⁵¹Cr release by H-2^k alloantiserum before absorption was calculated as 100% cytotoxic activity (Antibody mediated ⁵¹Cr release ranged from 40–65% of total incorporated chromium). Controls included spontaneous lysis of targets alone, lysis in presence of complement alone, H-2 antibodies alone, normal mouse serum alone and normal mouse serum together with complement.

Absorption studies were performed to quantify the possible H-2 alteration seen in immunofluorescence. Different numbers of viable infected and non infected L-cells (2.5 × 10⁷–1 × 10⁸) were incubated with 0.5 ml anti-H-2^k serum for 30 min at 37 °C. After this, the cytotoxic titre of the absorbed serum was tested in the ⁵¹Cr-release assay using C3H spleen lymphocytes (H-2^k) as targets and guinea pig serum as complement source (Figs 1 and 2). Then the lysis of target cells was assayed by Trypan blue exclusion using rabbit complement. Both tests gave nearly the same results.

Serum D-3b was absorbed quantitatively five times, with comparable results. There was a constant reduction of H-2 alloantiserum absorbing capacity of L-929 cells infected with vaccinia virus (Fig. 1). Using 1.0 × 10⁸ normal cells for absorption we found no cytotoxicity in the ⁵¹Cr-release assay. The complete absorption was not simulated by anti-complementary activity of the H-2 serum since the absorbed serum showed no more H-2 fluorescence, and absorption with increasing amounts of normal L cells showed a stepwise decrease of cytolytic activity. Serum absorbed with the same amount of infected cells showed cytolytic activity up to a serum dilution of 1:128. After absorption with 2.5 × 10⁷ and 5 × 10⁷ cells the anti-H-2^k serum showed 20–40% more cytolytic activity when infected cells were used for absorption (Fig. 2).

Table 1 Blocking of CMC against target cells infected with vaccinia virus

Lymphocytes*	Target cell	Cr release†	Specific lysis‡
Normal DBA/2	L-929	20.1 ± 1.2	
Normal C3H	not infected	18.5 ± 0.7	
DBA/2 sensitised to H-2 ^k		64.4 ± 0.8	44.3§
C3H sensitised to vaccinia virus		19.4 ± 1.3	
Normal DBA/2	L-929	21.2 ± 0.9	
Normal C3H	vaccinia virus infected	23.4 ± 0.8	
DBA/2 sensitised to H-2 ^k		22.5 ± 0.7	
C3H sensitised to vaccinia virus		72.8 ± 1.4	49.4§

*Lymphocytes from a pool of six mice per group. Virus sensitisation was performed by intraperitoneal injection of 1 ml 1 × 10⁶ TCID₅₀ ml⁻¹ vaccinia virus six days previously. Sensitisation to H-2^k was achieved by intraperitoneal injection of 1 × 10⁸ C3H spleen lymphocytes 10 d previously. Killer-target cell ratio, 100:1.

†Mean of percentage ⁵¹Cr release ± s.e.m. of 5 wells per group.

‡Specific lysis (%) = (⁵¹Cr release (%) by sensitised lymphocytes) – (⁵¹Cr release (%) by normal lymphocytes).

§Differences are statistically significant (P < 0.001).

The influence of target-cell infection on CMC was tested. Since the effector phase of the allograft reaction, which is reflected *in vitro* by the CMC of allogenic cells, is directed mainly against serologically defined (SD) antigens¹⁰, modifications of these antigens should influence the degree of CMC. DBA/2 mice (genotype H-2^k) were sensitised to H-2^k by intraperitoneal injection of 1×10^8 spleen cells from C3H mice. Ten days later, the DBA/2 lymphocytes were collected and the cytolytic activity of the CTL tested against infected and non-infected L-929 target cells in a ⁵¹Cr-release assay³. Table 1 shows the results of an experiment representative of the four carried out. After incubation of the CTL sensitised to H-2^k with the normal L cells we found a specific release of 44.3% of the incorporated chromium. There was, however, no specific lysis of the allogenic target cells infected corresponding to the serum absorption assays with vaccinia virus. In the immunofluorescence control the target cells had vaccinia virus surface antigen fluorescence and altered H-2 fluorescence as described above. In spite of serologically demonstrable H-2 antigens, the CTL could not kill the infected allogenic cells. With syngeneic attacker cells sensitised to vaccinia virus we observed significant specific lysis of the infected target cells.

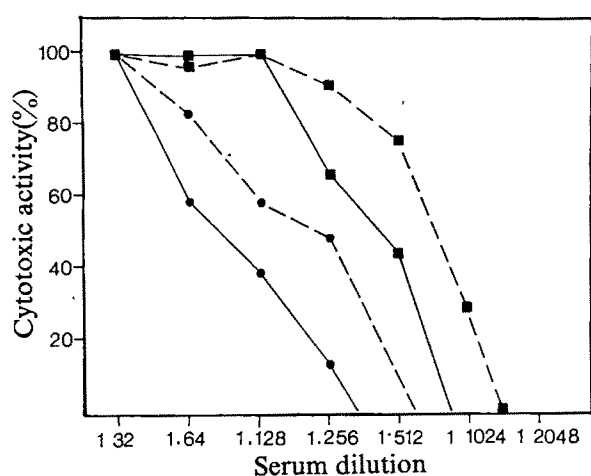


Fig. 2 Comparison of quantitative absorption by different numbers of vaccinia virus infected and normal L-929 cells. ●—●, H-2^k (0.5 ml) absorbed on 0.5×10^8 L cells; ○—○, 0.5×10^8 vaccinia virus infected L cells; ■—■, 0.25×10^8 L cells; □—□, 0.25×10^8 vaccinia virus infected L cells. Maximal ⁵¹Cr. release by H-2^k antiserum before absorption was calculated as 100% cytotoxic activity.

These data indicate that infection of L-929 fibroblasts with vaccinia virus may result in a reduction and an alteration of H-2 antigenic sites. It has been shown by serum absorption tests that tumour viruses can increase or diminish the level of H-2 specificities^{11,12}.

H-2 antigen expression is cell cycle dependent¹³ and possibly the quantitative alteration after vaccinia virus infection could be caused by a virus-induced nonspecific blocking of the metabolism of membrane proteins. Using enteroviruses as infective agents we were not able to find a reduced H-2 allo-anti-serum absorbing capacity or an inhibition of lysis of allogeneically infected cells in the CMC. Expression of viral surface antigens seems, therefore, to be a prerequisite for antigenic alteration. Rearrangement of H-2 antigen specificities¹⁴ accompanied by virus-induced inhibition of membrane fluidity does not seem to be a sufficient explanation for the inhibition of T-cell effector function, since antigenic recognition is possible after fixation of cells¹⁵.

If the expression of H-2 in infected and non-infected cells were identical by serological means and infection induces only quantitative differences of H-2 antigen expression, the cells should be lysable in the CMC against alloantigens¹⁶. There is, however, not only slight reduction, but complete blocking of CMC. This could mean that there is a virus-induced qualitative

change of H-2 antigenicity or a steric hindrance of the target antigen for the T cell sensitised to alloantigen. Different localisation of serologically and cellularly detectable antigenic sites on the same molecule could also explain the different results of antibody binding and allogenic CMC. The latter possibility is supported by the finding that CMC between cells identical in SD antigens and different only in H-2 allo-anti-serum absorbing capacity occurs¹⁷⁻¹⁸. Reduction of CMC against allogenic cells has been obtained by Gardner *et al.*²⁰ in the ectomelia system while modification of cells with TNP did not alter CMC to H-2 alloantigens⁵. These contradictory results may reflect the different sizes of the modifying agents or the different process and extent of alteration.

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Modulation of macrophage Fc receptor expression *in vitro* by insulin and cyclic nucleotides

THE capacity of certain cells to bind antibody or immune complexes through receptors at the cell surface may be important in both inductive and effector limbs of the immune response. Macrophages¹, B lymphocytes², activated T lymphocytes³ and certain tumour cells⁴ express such receptors which interact with the Fc portion of IgG (Fc receptors). I have shown that guinea pig macrophages are heterogeneous in their capacity to bind antibody, that the expression of Fc receptors is greatly increased during cell activation *in vivo* and *in vitro* and that this activation of receptor expression is inhibited by a factor(s) present in fresh autologous serum⁵. Here I show that changes in macrophage Fc receptor expression *in vitro* are modulated by insulin and by cyclic nucleotides.

Normal peritoneal cells were extracted aseptically from untreated outbred guinea pigs by washing the peritoneal cavity with 25 ml of RMPI 1640 tissue culture medium supplemented with 10% foetal calf serum (FCS) and containing heparin (5 U ml^{-1}). Samples (1 ml) of this suspension, which contained approximately 1×10^6 macrophages per ml, were placed immediately in each chamber of dual tissue culture slides (Lab-Tek Products). Cells from more than one animal were never pooled. These cells were incubated at

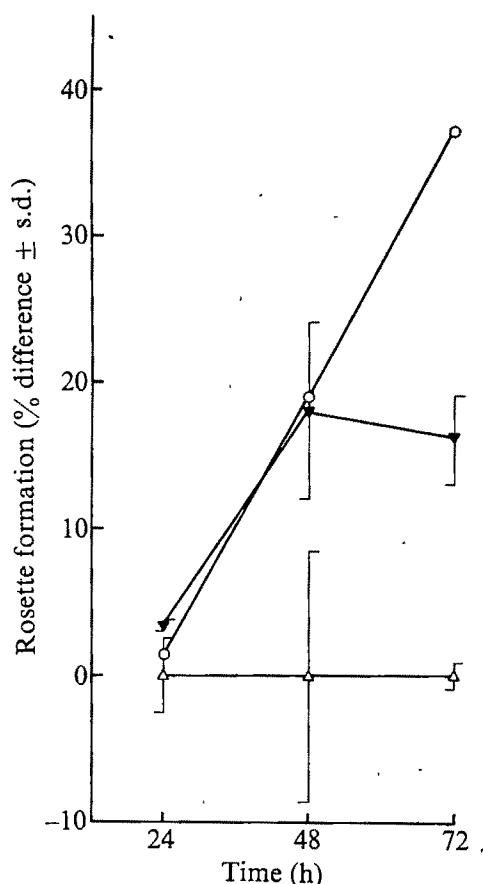


Fig. 1 The data are normalised by taking the percentage rosette formation in cultures exposed to insulin at 50 µg ml⁻¹ (△) as a reference and plotting the difference between these values and values in control cultures (○) and cultures exposed to insulin at 10 µg ml⁻¹ (▼). Each point represents the mean ± s.d. of three independent experiments.

37 °C for 2.5 h after which non-adherent cells were removed by washing the monolayer twice with heparin-free medium. The resultant monolayers, which consisted of macrophages of >95% purity, were cultured in RPMI 1640 supplemented with 20% FCS in an atmosphere of 5% CO₂ in air at 37 °C. In all cultures the medium was replaced every 24 h with a fresh preparation. Control cultures received RPMI with 20% FCS whereas experimental cultures received the same medium containing one of the following: insulin, dibutyryl cyclic AMP, dibutyryl cyclic GMP, 3-isobutyl-1-methyl-xanthine (IBMX), dibutyryl cyclic AMP+IBMX, dibutyryl cyclic GMP+IBMX, at the concentration specified.

Guinea pig IgG anti-sheep erythrocyte antibody was prepared from the sera of animals mounting a secondary response to sheep erythrocytes in Freund's complete adjuvant⁵. Antibody-sensitised sheep erythrocytes (EA) were prepared by incubating the cells with various concentrations of this antibody, and a degree of EA sensitisation appropriate for monitoring changes in macrophage Fc receptor activity was determined by obtaining a dose-response curve for rosette formation on monolayers at day 0 of culture, as described previously⁵. On subsequent days of culture the percentage rosette formation in control cultures in one chamber of a dual chamber slide was compared with that in experimental cultures in the other chamber. Thus, after rosette formation with a single preparation of EA, cultures were washed three times in Hanks' balanced salt solution, fixed with 1% glutaraldehyde and stained with buffered Giemsa. Five hundred cells were counted per culture and macrophages bearing five or more erythrocytes were scored as rosettes and expressed as a percentage of the total cells.

In control cultures the percentage rosette formation increased markedly as expected during 72 h of culture. When cells were cultured in medium containing 10 µg ml⁻¹ or 50 µg ml⁻¹ of insulin, however, the increase in receptor expression was inhibited to a degree related to the concentration of insulin present (Fig. 1). Existing Fc receptors were not blocked by insulin, but the increase in receptor expression was inhibited in a dose related manner. The effect therefore is unlikely to be due to steric hindrance of Fc receptors by surface-bound insulin. This *in vitro* effect resembles that of fresh autologous serum in that activation is inhibited⁶. Insulin did not significantly affect cell viability. When cells were cultured in the presence of dibutyryl cyclic AMP (5 × 10⁻⁵ M) the increase in receptor expression was again inhibited (Fig. 2). IBMX at the same concentration also inhibited the change in receptor expression. IBMX is a potent inhibitor of phosphodiesterases which hydrolyse cyclic AMP to form 5'-AMP (ref. 6); its presence should thereby cause an accumulation of intracellular cyclic AMP. When both dibutyryl cyclic AMP and IBMX were present the increase in receptor activity was inhibited to a greater degree than when either was present alone (Fig. 2). In the latter cultures Fc receptor activity remained at about the same level as that initially present. This synergistic effect would be expected to result from inhibition of the hydrolysis of both intracellular cyclic AMP

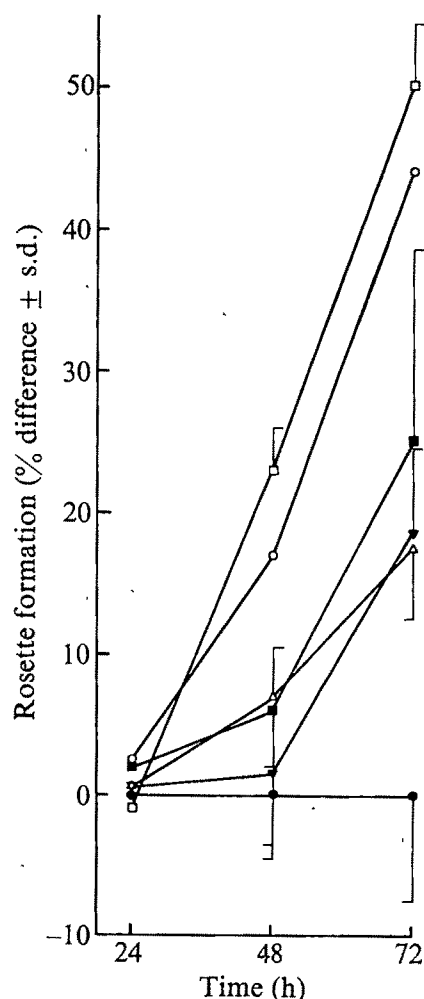


Fig. 2 The data are normalised by taking the percentage rosette formation in cultures exposed to 5 × 10⁻⁵ M dibutyryl cyclic AMP + 5 × 10⁻⁵ M IBMX (●) as a reference and plotting the difference between these values, control values (○), and values in cultures exposed to the following: dibutyryl cyclic AMP (△), dibutyryl cyclic GMP (□), IBMX (▼), dibutyryl cyclic GMP + IBMX (■) all at a concentration of 5 × 10⁻⁵ M. Each point represents the mean ± s.d. of four independent experiments. For clarity only one s.d. is shown on each point.

and dibutyryl cyclic AMP added to the medium. It further indicates that cyclic AMP, rather than 5'-AMP or other catabolites, exerts the observed effect. Dibutyryl cyclic MP (5×10^{-5} M) slightly augmented the increase in receptor expression (Fig. 2). When both dibutyryl cyclic GMP and IBMX were present at the same concentration the inhibitory effect of IBMX prevailed (Fig. 2). Presumably the effect of accumulated intracellular cyclic AMP is more telling than that of dibutyryl cyclic GMP added to the extracellular medium. Cell viability did not differ significantly between control and experimental cultures. Dibutyryl cyclic AMP at a concentration of 5×10^{-6} M had no effect on receptor expression (data not shown).

Cyclic nucleotides have been implicated in the control of various immunological and inflammatory functions⁷. In general cyclic AMP inhibits and cyclic GMP augments such functions as T-lymphocyte cytotoxicity⁸, lymphocyte proliferation and antibody production⁹, histamine release by mast cells¹⁰, lectin-induced uptake of calcium by T lymphocytes¹¹. My results are compatible with a model of macrophage activation in which the expression of Fc receptors is a function of antagonistic signals: insulin or hormones resembling insulin exerting a stabilising influence by maintaining a high intracellular cyclic AMP: cyclic GMP ratio, and other signals, possibly lymphokines, exerting an activating influence by lowering this ratio. The least requirement for activation *in vitro* may be the removal of the stabilising influence. It is interesting that a receptor for insulin has recently been demonstrated on human monocytes (R. H. Schwartz, A. R. Bianco, B. S. Handwerker, and C. R. Kahn, unpublished). A similar mechanism may also control T-lymphocyte Fc receptor expression which resembles that of macrophages⁵ in so far as the development of receptors during culture occurs in FCS but not in autologous serum¹². The expression of Fc receptors on cells derived from a murine myeloma has been shown to be inhibited by cholera exotoxin which increased intracellular cyclic AMP levels¹³.

An understanding of the mechanism controlling macrophage Fc receptor expression is important because changes in such expression may have a regulatory role both in the induction of an immune response and in the effector mechanisms of immunological surveillance. Increased receptor expression may facilitate the presentation of antigen to T lymphocytes since macrophage-bound antibody can mediate such presentation^{14,15}. It may also facilitate the killing of target cells by macrophages and I suggest that this explains the increased antibody dependent cytotoxicity exhibited by cultured macrophages¹⁶. Since circulating tumour antigen or antigen-antibody complexes seem to suppress the cellular response against tumours¹⁷, increased macrophage Fc receptor expression might also be expected to facilitate this response by accelerating the clearance of immune complexes.

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Molecular differences of exposed surface proteins on thrombasthenic platelet plasma membranes

THE platelet plasma membrane contains several proteins of different molecular weights. Three of these proteins stain for carbohydrate with periodic acid-Schiff reagent and have been termed glycoproteins I, II and III (molecular weights 150,000, 124,000 and 106,000, respectively)^{1,2}. It has been suggested that the bleeding tendency observed in Glanzmann's thrombasthenia results from abnormalities of the platelet membrane³⁻⁶. Nurden and Caen⁷ showed that glycoprotein II was absent in a crude membrane fraction isolated from thrombasthenic platelets. We have now investigated the surface composition of thrombasthenic membranes by the lactoperoxidase iodination technique² and found a low concentration of glycoprotein II, and other molecular differences.

When normal platelets are iodinated by the lactoperoxidase technique, glycoproteins I, II and III are labelled together with numerous other polypeptides of lower molecular weight². In addition a membrane component is labelled that has a molecular weight between that of glycoproteins I and II (refs 8 and 9). It is proposed that this component, which stained for carbohydrate, albeit poorly, be termed glycoprotein IIa while the former glycoprotein II be termed glycoprotein IIb. Figure 1 shows the iodination patterns from two normal donors.

The platelets from three patients⁸ fulfilling the diagnostic criteria of Glanzmann's thrombasthenia were iodinated with ¹²⁵I (CEA, Saclay, France), solubilised and mixed with solubilised normal platelets previously labelled with ¹³¹I. The mixtures were reduced with 2% 2-mercaptoethanol and submitted to sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis². The iodination patterns of the platelets from the three patients were almost identical and revealed marked differences from normal in the glycoprotein region. Figure 1a shows the iodination patterns obtained after coelectrophoresis of normal platelets with those from one of the patients. These patterns revealed an increase in the label associated with a polypeptide with an apparent molecular weight similar to glycoprotein I, a normal label associated with glycoprotein IIa and a decreased label associated with glycoproteins IIb and III. In addition, the molecular weight of thrombasthenic glycoprotein III was less than that of normal glycoprotein III. The ratio of the label associated with the glycoprotein region of thrombasthenic platelets from the three patients studied was 1.5:1.0:4.0:5, whereas that associated with the glycoproteins of normal platelets was 1:1:1:3.

The plasma membranes from iodinated thrombasthenic platelets were isolated by the glycerol-lysis technique¹². Thrombasthenic platelets lysed equally as well as normals and gave yields of plasma membranes within the normal range. Isolated membranes were then coelectrophoresed with normal iodinated platelets (Fig. 1b). A difference was observed between the iodination patterns of thrombasthenic platelets and isolated membranes from these platelets. Comparison of Fig. 1a and b shows that the labelled component of thrombasthenic platelets with an apparent mole-

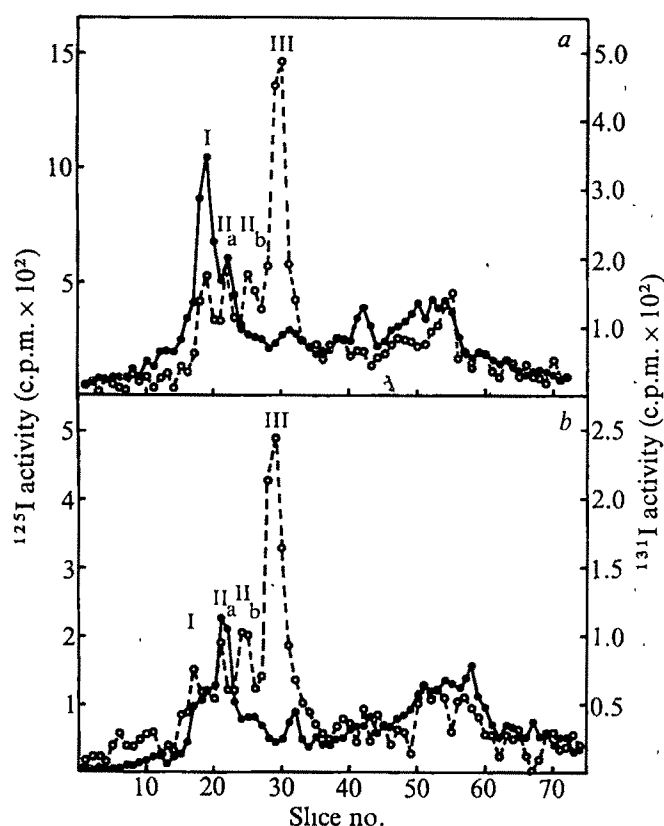


Fig. 1 Comparison of the exposed polypeptides and glycoproteins of the plasma membranes of normal platelets and platelets from a patient with Glanzmann's thrombasthenia. Blood from a normal donor and the patient were anticoagulated using ACD (acid citrate dextrose)¹⁰ and the platelets were isolated and washed by the method of Massini and Lüscher¹¹. Washed normal platelets (1 ml, 10^9 platelets) were labelled with ^{131}I whereas the washed platelets from the patient (1 ml, 10^9 platelets) were labelled with ^{125}I by the lactoperoxidase technique⁹. Labelled platelets were washed twice and solubilised in 2–3% SDS. Labelled membranes from the patient's platelets were also isolated¹² and solubilised in 2–3% SDS. Samples labelled with ^{125}I were mixed with those labelled with ^{131}I . These mixtures were treated with 2% 2-mercaptoethanol, heated to 100 °C for 3 min, and submitted to electrophoresis on 5% polyacrylamide gels, sliced laterally into 1.6-mm disks and the radioactivity associated with each disk determined⁸. *a*, ^{125}I -labelled thrombasthenic platelets (●) and ^{131}I -labelled normal platelets (○); *b*, ^{131}I -labelled normal platelets (○) and plasma membranes from ^{125}I -labelled thrombasthenic platelets (●).

cular weight similar to that of glycoprotein I was not isolated with the plasma membranes. Further analysis revealed that this component was also not present in the residue fraction, but appeared in the supernatant. Normal glycoprotein I, however, is isolated with the plasma membrane fraction in a concentration similar to that present in whole platelets². It therefore seems that the labelled component in the glycoprotein I region of thrombasthenic platelets, which is absent on normal platelets, is not glycoprotein I but rather a component bound to the thrombasthenic membrane surface. The significance of the decreased label associated with thrombasthenic glycoprotein I is not clear since gels stained for carbohydrate of isolated thrombasthenic membranes (not shown) revealed that the concentration of glycoprotein I was within the normal range.

Another difference between normal and thrombasthenic platelets is the decreased iodination of glycoprotein IIb on the thrombasthenic platelet membrane. Nurden and Caen⁷ concluded from studies of gels of crude thrombasthenic membranes stained with periodic acid-Schiff that glycoprotein II (glycoprotein IIb) was absent. Staining of gels

for carbohydrate, however, cannot detect low concentrations of this component. In the study reported here, because of the greater sensitivity of the iodination technique, the amount of label associated with glycoprotein IIb was 40% of that present on the normal platelet membrane. We suggest therefore that the phenotypic expression of thrombasthenia is not an absence of glycoprotein IIb but rather a reduction in its concentration.

The study reported here demonstrates that glycoprotein III is also abnormal in Glanzmann's thrombasthenia. Nurden and Caen's results⁷ indicated that thrombasthenic glycoprotein III had a lower apparent molecular weight but that its concentration was similar to that of normal platelets. The data from the iodination experiments reveal that the decreased molecular weight of thrombasthenic glycoprotein III has drastically altered its expression on the membrane surface. The decreased iodination of thrombasthenic glycoprotein III may result from either an altered primary structure which could affect its orientation in the membrane or from a missing peptide containing a tyrosine residue which is iodinated in normal glycoprotein III. Since an additional component is found on the thrombasthenic platelet membrane surface it is possible that it sterically hinders the iodination of glycoprotein III.

Platelets from patients with Glanzmann's thrombasthenia do not aggregate to adenosine diphosphate (ADP), collagen or thrombin, even though collagen and thrombin induce thrombasthenic platelets to undergo a release reaction similar to normal platelets^{3–6}. Although ADP does not induce aggregation of these platelets, it does stimulate the platelet shape change⁴. Therefore, it seems that receptors for ADP, collagen and thrombin are present on the thrombasthenic platelet membrane but that these platelets lack an essential component necessary for normal aggregation. Since platelet aggregation involves interactions between membrane surfaces, the differences in the surface proteins of thrombasthenic platelets seen in our study could account for the absence of aggregation in this disorder and may indicate those components involved in normal ADP-induced aggregation.

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Actin-tubulin homology revisited

THERE seems to be a principle underlying all eukaryotic motile systems. Movement, whether it be muscular, ciliar, flagellar or cytoplasmic, is apparently realised through the ATP-dependent sliding interaction of fibrillar proteins. This has raised the controversial question of whether the corresponding proteins from different systems are in fact homologues. Indeed, many proteins involved in motility have been isolated and characterised and have been reported as actins¹⁻⁵.

Evidence has been presented for^{6,7} and against^{8,9} the concept of homology between actin and tubulin, the monomeric unit of microtubules. (We use 'homology' in the sense defined by Stephens⁸.) Puszkín *et al.*^{10,11} demonstrated that colchicine-binding protein isolated from porcine brain¹⁰ and blood platelets¹¹ can stimulate ATPase activity of myosin in much the same way as actin. Others however, have shown large quantities of actin-like protein in these tissues^{12,13}, and so the results of Puszkín *et al.* might have been affected by contaminating actin.

The outer fibres of the ciliary axoneme provide an ideal tool for corroborating Puszkín and Berl's findings for large quantities of pure tubulin can be obtained easily⁶. There is no evidence that cytoplasmic actin is present in cilia of *Tetrahymena pyriformis*¹⁴.

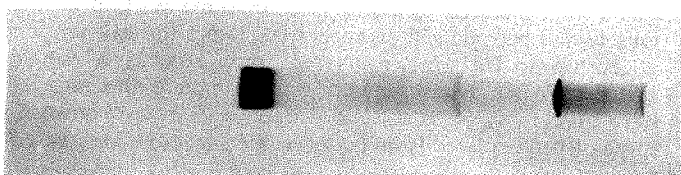
Cilia of *T. pyriformis* (W strain) were isolated and purified as before⁶. Outer fibres were prepared by dialysing demembrated axonemes against a low ionic strength buffer (1 mM Tris-thioglycollate, 0.1 mM EDTA). The outer fibre fraction was dissolved in a solution containing the detergent Sarkosyl (0.5% Sarkosyl; 10 mM imidazole-HCl, pH 6.8; 100 mM KCl; 0.2 mM GTP and 0.1% mercaptoethanol) followed by Sephadex G-25 column chromatography to remove unbound detergent. The homogeneity of the preparation was determined by polyacrylamide gel electrophoresis¹⁵.

Chicken breast myosin was isolated by the method of Richards *et al.*¹⁶. The concentration of both tubulin and myosin was determined by the technique of Lowry *et al.*¹⁷, using bovine serum albumin as a standard. The ATPase activity of myosin, in both the presence and absence of outer fibre tubulin, was measured using the Fiske-Subba Row¹⁸ assay for inorganic phosphate (for details see Table 1).

The results are shown in Table 1 and Figs 1 and 2. Figure 1 illustrates the homogeneity of the preparation, for essentially only the two bands of A and B tubulin are present in the stained polyacrylamide gel. Table 1 summarises the effect of the addition of tubulin on the Mg²⁺ ATPase activity of myosin. Activity increased about 3.8 ± 0.4 times. The tubulin-myosin ratio was not critical; optimal stimulation of activity was at a ratio of 3:1 or greater. The trace amount of ATPase activity associated with the tubulin fraction probably represents residual dynein¹⁹.

The effect of changes in the pH of the reaction mixture

Fig. 1 Polyacrylamide gel electrophoresis of outer fibre tubulin from the cilia of *Tetrahymena*. The protein was reduced and alkylated as before⁶, and run in 5% acrylamide gels with 8 M urea¹⁵. Approximately 70 µg of protein were loaded per gel.



on the tubulin stimulation of myosin is illustrated in Fig. 2. The pH curve for the interaction shows an optimum at 6.0, decreasing sharply on either side (Fig. 2), thus emphasising the importance of pH in the tubulin-myosin interaction.

These results essentially agree with those of Puszkín *et al.*^{10,11}, but differ in one important aspect. They reported more than 20-fold stimulation of myosin activity with porcine brain¹⁰ and platelet¹¹ tubulin at pH 6.8 whereas we found a roughly two-fold stimulation at that pH (Fig. 2). An explanation for the lower stimulation may be a partial denaturation of the ciliary tubulin by Sarkosyl. Data indicate that muscle actin solubilised in Sarkosyl loses 70% of its ability to stimulate the ATPase activity of myosin

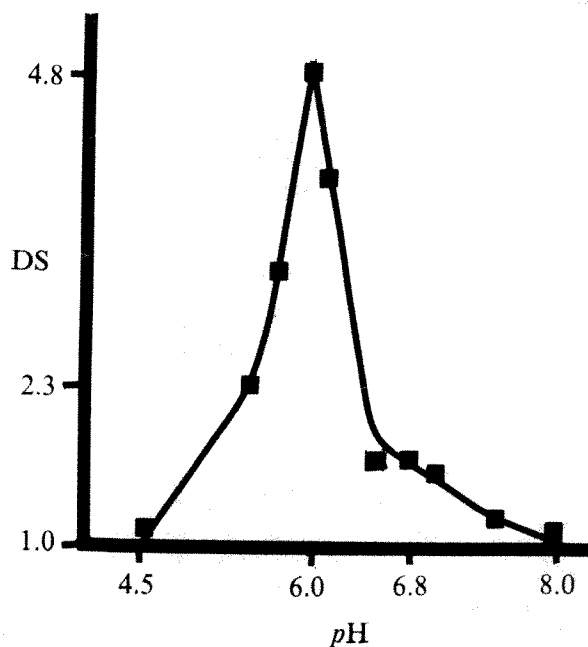


Fig. 2 Effect of pH on the stimulation of myosin ATPase by outer-fibre tubulin. The assay was performed as described in Table 1, except that the buffer used for pH values above 7 was Tris-HCl. Each point represents the average of at least two experiments. The degree of stimulation (DS) was calculated from the following formula: $DS = (MT - T)/M$ where M , T , and MT represent the specific activities of myosin, tubulin and myosin plus tubulin respectively. Note that a DS value of 1.0 represents no stimulation.

(unpublished work). On the other hand, a survey of the literature indicates that actin from different sources stimulates myosin ATPase activity to different degrees^{3,5}. If this is taken into consideration the value we obtained is comparable with that reported for the stimulation of muscle myosin by *Acanthamoeba* actin³ where the stimulation was approximately fourfold.

The optimal pH of the interaction (6.0) agrees rather well

Table 1 Stimulation of Mg²⁺ ATPase activity of muscle myosin by ciliary outer fibre tubulin.

Protein sample	Specific activity (µmol Pi per min per mg protein)
Myosin	0.028 ± 0.06
Tubulin	0.005 ± 0.01
Myosin and tubulin	0.097 ± 0.05

The reaction mixture consisted of 30 mM imidazole-HCl buffer, pH 6.0, at 25 °C; 0.06 M KCl; 0.5 mM ATP and 1 mM MgCl₂. Each tube contained 2 ml of reaction mixture plus 40 µg of myosin and/or 114 µg of tubulin. Duplicate samples were incubated in a water bath and stopped after 0 and 30 min incubation by the addition of 0.5 ml of sodium dodecyl sulphate, followed by 0.25 ml of molybdate reagent and 0.15 ml of the Fiske-Subba Row reagent. The tubes were then read at 660 nm with a Zeiss PMQ-11 spectrophotometer.

with the reported pK of histidine. This suggests that histidine and its methylated derivative in actin could play a role in the stimulation of the myosin ATPase activity by both actin and tubulin. This is supported by the finding that histidine is essential for the polymerisation of G-actin²⁰ together with the report that polymerisation is characteristic of the interaction between G-actin and myosin²¹.

The idea of homology between actin and tubulin arose from the finding that the amino acid composition and molecular weight of tubulin closely parallel those reported for actin⁶. Other workers questioned this homology. Mohri and Shimomura reported no stimulation of myosin by sea urchin sperm flagella⁹. However, they solubilised the tubulin in thermal conditions that could result in the denaturation of the protein²². Stephens⁸ bases his objections to the homology on the fact that the newer determination of the molecular weight of actin²³ does not agree with that established for tubulin. Indeed, he shows that the peptides produced by tryptic and chymotryptic digests of tubulin and actin isolated from *Pecten irradians* are dissimilar. There may however, be limited regions in the primary and tertiary structure of tubulin and actin that are similar, which could explain the stimulation of myosin by tubulin.

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Cross innervation and the regulatory protein system of rabbit soleus muscle

SINCE Buller *et al.* demonstrated¹ that the contraction speed of skeletal muscle is determined by the nature of its innervation, there have been several reports that the biochemical composition and properties of muscle are likewise influenced. When the motor nerve to a slow muscle is replaced by a nerve that normally innervates a fast muscle, changes in phosphorylase, succinic dehydrogenase and myosin ATPase activities, such as would be expected if the biochemical characteristics of the fibres were changing from those of type I to those of type II, can be demonstrated histochemically². This is also true in the opposite situation, when the nerve to a fast muscle is replaced with one that normally innervates a slow muscle. The histochemical evidence implies that a change in concentration of the enzymes studied has occurred in response to the new type of innervation and although it would not be evident in such investigations, the possibility of change in isoenzyme complement cannot be excluded. In the case of myosin, however, which has been isolated after cross innervation, the changes

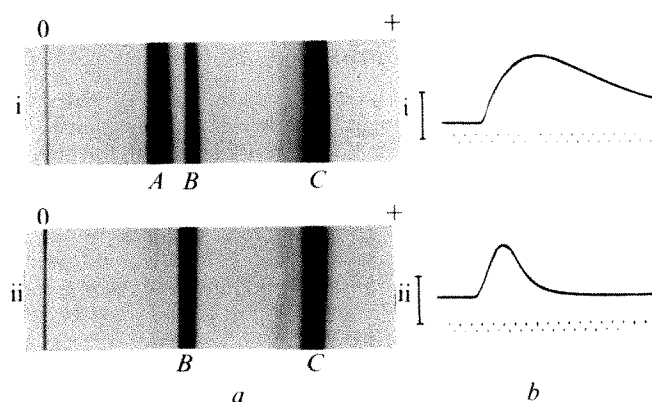


Fig. 1 Effect of cross innervation on the speed of contraction and the troponin I composition of rabbit soleus muscle. *a*, Electrophoresis of troponin I (20 μ g), isolated by affinity chromatography from rabbit soleus muscle (rabbit 2, see Table 1), in the presence of troponin C (50 μ g) from rabbit white skeletal muscle. 6 M urea, 25 mM Tris–80 mM glycine, pH 8.6, 8% acrylamide. O, Origin. i, Control soleus; ii, soleus from the other leg of the same animal 26 weeks after cross innervation. A, slow troponin I–troponin C complex; B, fast troponin I–troponin C complex; C, troponin C. *b*, Records of single isometric contractions of the soleus muscles used for troponin I preparations analysed in *a*. The distance between successive dots represents 10 ms. Vertical scale represents 100 g tension.

have been analysed with more precision. These indicate that when the contraction speed of skeletal muscle is altered by either cross innervation or long term electrical stimulation, there are changes in V_{max} of the myosin ATPase, in its light chain components and in its electrophoretic mobility^{3–7}. These changes imply that new myosin molecules of different primary and quaternary structure are being synthesised as a consequence of the altered activity pattern of the muscle resulting from these procedures. They correlate well with the widely accepted view that the V_{max} of the myosin ATPase rises with increasing muscle speed⁸ and the differences in light chain components known to exist in myosin from fast and slow skeletal muscle^{9–12}.

Biochemical investigations carried out so far on cross-innervated muscles have been restricted to enzymes involved in energy production and utilisation. As there is now good evidence that tropomyosin¹³ and the components of the troponin system differ in fast and slow skeletal muscle (see ref. 14 for review), we have investigated the effect of cross innervation on those proteins of the I filament which are involved in the regulation of the actomyosin ATPase.

Cross innervation of the soleus muscle was performed in 13 adult New Zealand Red rabbits under Nembutal anaesthesia. Using full aseptic precautions, the nerve to the soleus muscle in one leg was sectioned and sutured to the medial head of the gastrocnemius muscle to prevent self reinnervation. The nerve to the fast muscle, the lateral popliteal, was cut close to its point of entry in to the tibialis anterior muscle and sutured to the soleus. Between 2 and 6 months later, the soleus muscles of both operated and control legs were prepared for the recording of isometric tension¹⁵. Control muscles were stimulated by way of the cut peripheral end of the medial popliteal nerve with square-wave pulses of supramaximal intensity. Contractions of the cross-innervated muscles were elicited by stimulation of the lateral popliteal nerve. The medial popliteal nerve was also stimulated to assess whether some portion of the muscle had become self reinnervated. Times to peak tension of both muscles were measured from records of single twitches. When measurements were complete the muscles were frozen at -20°C and troponin I or tropomyosin subsequently isolated from both control and cross-innervated muscles.

For preparation of troponin I the muscles were homo-

Table 1 Effect of cross innervation on the relative proportions of the polymorphic forms of troponin I and tropomyosin in rabbit soleus muscle

Animal no.	Weeks after cross innervation	Cross innervated soleus					Control soleus			
		Contraction time (ms) X Own nerve	Contraction time (ms) nerve*	Tropomyosin α/β (molar ratio)	Troponin I		Contraction time (ms)	Tropomyosin α/β (molar ratio)	Troponin I	
					Fast (%)	Slow (%)			Fast (%)	Slow (%)
9	8	62	—	—	50	50	70	—	31	67
8	8	32	—	—	73	27	61	—	24	76
3	22	32	63	—	16	84	54	—	30	70
5	23	29	—	—	77	23	75	—	36	64
2	26	26	—	—	97	3	52	—	37	63
1	27	25	—	—	79	21	54	—	27	73
10	12	31	—	1.10	—	—	60	1.04	—	—
11	13	44	61	1.07	—	—	54	0.98	—	—
12	19	30	—	1.06	—	—	70	1.00	—	—
14	20	30	—	0.96	—	—	80	1.03	—	—

*The absence of figures in this column indicates that reinnervation by the muscle's own nerve did not occur.

Relative amounts of the fast and slow forms of troponin I and of the α and β subunits of tropomyosin were determined by comparing the intensities of staining with Coomassie brilliant blue of electrophoretic bands, using a Unicam SP500 spectrophotometer coupled to a Gilford linear transporter absorbance indicator. Electrophoresis of troponin I carried out in the conditions described in text and in Fig. 1; tropomyosin applied to a 10% (w/v) polyacrylamide gel with 0.1% SDS, 6 M urea, and 82.5 mM Tris-400 mM boric acid buffer, pH 7.0 (ref. 13).

genised in 20 vol of a solution containing 9 M urea, 1 mM CaCl_2 , 15 mM 2-mercaptoethanol, 75 mM Tris, adjusted to pH 8.0 with 1 M HCl, and applied to a Sepharose-troponin C affinity column as described¹⁰. After washing well with buffer and subsequent application of the same buffer in which the CaCl_2 was replaced by 10 mM EGTA, troponin I was eluted from the column free from other proteins.

Two polymorphic forms of troponin I are present in rabbit skeletal muscle. These are fast skeletal troponin I, which is the only form present in psoas and longissimus dorsi muscle¹⁰ and slow skeletal troponin I, which is the principal form present in red skeletal muscles such as the soleus and crureus. The two forms have different primary structures but migrate with identical mobilities on electrophoresis in SDS. Although neither form of troponin I migrates to the anode on electrophoresis in 6 M urea, 25 mM Tris-80 mM glycine, pH 8.6, the complexes formed by the two forms with troponin C in the presence of Ca^{2+} in these conditions show electrophoretic mobilities sufficiently different to allow them to be distinguished readily and their relative amounts estimated (Fig. 1). Control experiments using a sample of pure fast skeletal troponin I to which 3-4 times a molar excess of troponin C isolated from white rabbit skeletal muscle was added, showed that all the troponin I present in the mixture moved as the complex on electrophoresis in 6 M urea, pH 8.6. Thus by densitometric scanning of the bands of the complexes formed on electrophoresis when the total troponin I extracted from the muscle was mixed with excess troponin C, an estimate of the relative amounts of the two forms of troponin I present could be made. The validity of this procedure to within $\pm 3\%$ was confirmed by control experiments in which samples of troponin I of known composition were applied to the gel. In this way, we have shown that the troponin I of normal New Zealand Red rabbit soleus consists of about 30% fast and 70% slow skeletal troponin I.

In all cases in which the contractile speed of the soleus muscle increased after cross innervation, a corresponding change in the proportion of fast and slow troponin I was observed. On average, 22-27 weeks after cross innervation, the fast skeletal troponin I increased from 30% to more than 70% of the total troponin I extracted by affinity chromatography. In one experiment the troponin I present in the soleus was converted almost completely to the fast skeletal muscle type (Fig. 1 and Table 1, animal no. 2). Where physiological measurements showed that most of the muscle had become reinnervated by its own nerve (Table 1, animal no. 3), the speed of contraction remained slow

and there was no increase in the relative amount of fast troponin I. The change in the proportion of the two forms of troponin I seemed to precede the change in contractile speed in one animal (Table 1, animal no. 9) that was examined 8 weeks after the operation. This observation is being investigated further.

The form of troponin I that increased in amount was not a simple modification of the slow muscle type but represented new protein synthesis for the electrophoretic pattern of the cyanogen bromide digest was similar to that previously described for fast skeletal muscle troponin I of the rabbit and different from that of the cyanogen bromide digest of slow skeletal troponin I (ref. 16).

Tropomyosin was isolated from soleus muscles frozen immediately after measurement of the speed of contraction by the method of Cummins and Perry¹³. The protein was separated into the α and β components by electrophoresis, the bands stained with Coomassie brilliant blue and the relative amounts present determined by densitometric scanning¹³. A molar ratio of α and β subunits in soleus muscle of the New Zealand Red rabbit of 1.01 was obtained, which is similar to that reported for the New Zealand White rabbit¹³. The ratio did not change significantly 3-6 months after cross innervation, when the contraction time had increased by a factor of approximately two and marked changes in the troponin I composition had occurred (Table 1).

The evidence indicates that when the contractile speed of soleus muscle is increased by replacing its normal nerve supply by a nerve that usually supplies a fast muscle, the slow skeletal troponin I (which is the major polymorphic form of the protein present in whole soleus muscle) is replaced by fast skeletal muscle troponin I. If no changes in the relative rates of catabolism of these two proteins occur this implies that the change in innervation has altered the relative rates of synthesis of the two forms of troponin I, which, from their primary structural differences¹⁶, would be presumed to be under different genetic control.

Although the molar ratios of α and β subunits of tropomyosin in the muscles of the rabbit roughly correlate with the speed of contraction¹³ there was no change in this ratio during similar periods of cross innervation. Troponin C was not examined after cross innervation for there is evidence that this protein is very similar, if not identical, in fast and slow skeletal muscle^{17,18}. Neither was troponin T studied although different forms of this protein exist in the red and white skeletal muscle of the chicken^{19,20}. We conclude that the type of innervation markedly affects the polymorphic form of troponin I in the muscle, but not the

tropomyosin and probably not the troponin C. This implies that different factors are involved in regulating the genes concerned in the synthesis of the individual proteins of the regulatory system.

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New peptide in the vertebrate CNS reacting with antigastrin antibodies

RELEASING factors¹ and other brain peptides² have been the subject of extensive studies and the high sensitivity of modern measurement techniques has led to the discovery, in various tissues^{3,4} and in the nervous system⁵⁻⁸, of new, unpredicted locations for otherwise well known peptides. Evidence for the presence in the vertebrate nervous system of a hitherto undescribed brain gastrin immunoassayable peptide (BGP) is presented here.

Various tissues and regions of the central nervous system (CNS) were studied using human material obtained 24 h after death and animal material was dissected immediately after decapitation. All specimens were inspected under the microscope for normality. For extraction of immunoassayable material, a 10% w/v suspension of fresh tissue in a buffer of potassium phosphate (0.05 M) and sodium chloride (0.15 M) at pH 7.4 was homogenised at 4°C with a glass homogeniser and a motor-driven Teflon pestle. Samples (2 ml) of homogenate were used for dry weight analysis and the rest was boiled for 15 min and then centrifuged at 100,000g for 30 min. Samples of supernatant were taken for estimation of Lowry-positive material¹⁰ and of gastrin immunoassayable material using a technique with antigastrin antibodies of high specificity¹¹. Limits of detection were 15 or 60 pg Gastrin 2-17 (hexadecapeptide, ICI) per ml in 200-μl or 50-μl samples, respectively.

To obtain a dose-response curve, Gastrin 2-17 and boiled extract of human cortical grey matter concentrated after lyophilisation were used. For standard curve establishment, Gastrin 2-17 was diluted in a buffer of potassium phosphate (0.01 M) and sodium chloride (0.15 M) at pH 7.4 containing 10 mg per ml egg albumin (Sigma), or in human cortical grey matter boiled extract from which immunoreactivity had or had not been removed by charcoal absorption¹².

Our results for gastrin in blood serum are in agreement with those published¹³⁻¹⁵ and similar results were obtained for

gastric antrum^{16,17}, obtained from autopsy material. Distribution of BGP is otherwise restricted to the nervous system (Table 1). Highest concentrations are found in cortical grey matter of all cerebral lobes; concentration in the hypothalamus is much lower and similar to concentrations of brain stem and spinal cord. BGP was not detectable, in the peripheral nervous system, in cerebellum nor in any invertebrate nervous system studied so far (Table 2).

Dose-response curves (Fig. 1) with unlabelled Gastrin 2-17 are different from those obtained with successive dilutions of concentrated boiled cortical grey matter extract. When Gastrin 2-17 is diluted in boiled brain extract from which immunoreactivity has been removed by charcoal absorption¹² the dose-response curve is very similar to that obtained with regular diluant. When Gastrin 2-17 is added to immunoreactive boiled brain extract, a further decrease in the bound-to-free radioactivity ratio was obtained. On the contrary, however, the slope of the dose-response curve is less accentuated with concentrated boiled cortical grey matter extract, than with Gastrin 2-17, and with the highest concentrations the bound-to-free ratio rests near 0.10. These differences in dose-response curves were observed with two different rabbit antigastrin sera.

Following Feldman and Rodbard¹⁸, we can use the equation

$$R^2 + R(P'K + PK - QK + 1) - QK = 0 \quad (1)$$

(*R* is the bound-to-free ratio, *P'* the concentration of labelled Gastrin 2-17, *P* the concentration of unlabelled Gastrin 2-17, *Q* the concentration of antigastric antibodies sites, and *K* the antibody affinity constant at equilibrium for Gastrin 2-17) to calculate from the dose-response curve an affinity constant for Gastrin 2-17 of our antibody of 4.9×10^{10} l per mol Gastrin 2-17. This figure is similar to the values obtained by Rehfeld *et al.*¹⁸ which are between 0.5×10^{10} and 100×10^{10} l mol⁻¹ Gastrin 2-17. Similar calculations with BGP dose-response curve lead to an affinity constant of 0.9×10^{10} l mol⁻¹, showing clearly that BGP and Gastrin 2-17 are not identical competitors ($K_{\text{gastrin}} = 5.4 \times K_{\text{BGP}}$) with labelled Gastrin 2-17 for binding our antigastrin antibody. Whether these differences in affinity and the plateau obtained with highest concentrations of BGP are a result of the presence of different antigastrin antibodies at different concentrations, some reacting with Gastrin 2-17 and BGP, some reacting only with Gastrin 2-17, remain to be studied.

Disappearance of immunoreactivity of BGP, gastric antrum extract and Gastrin 2-17 was obtained with incubation at 37°C and pH 7.4 for 30 min with Pronase E (70,000 PUK g⁻¹, Merck) 1 mg ml⁻¹ or chymotrypsin A₄ (für analytische Zwecke, Boehringer) 1 mg ml⁻¹. With 1 mg ml⁻¹ trypsin (für analytische Zwecke, Boehringer), 70% of the immunoreactivity was preserved, as with gastric antrum extract or Gastrin 2-17. This is in accordance with the relative resistance to trypsin of Gastrin

Table 1 Distribution of gastrin immunoassayable substance in selected human tissues

Tissue	Dry weight	Lowry-positive material	Gastrin assay
Cerebral lobes	344 ± 3	21 ± 0.3	130 ± 7
Gastric antrum	302 ± 6	36 ± 1.3	14,800 ± 700
Blood serum	228 ± 3	25 ± 0.4	0.44 ± 0.05
Liver	357 ± 2	44 ± 1.1	< 3
Skeletal muscle	352 ± 6	43 ± 1.8	< 3
Myocardium	329 ± 2	41 ± 1.2	< 3
Lung	298 ± 4	47 ± 1.7	< 4
Kidney	293 ± 2	39 ± 1.1	< 4

Tissue dry weight in mg per g wet weight, boiled extract Lowry-positive material in mg per g dry weight, boiled extract gastrin immunoassayable material (gastrin assay) in ng per g dry weight. Means and standard errors of the mean (±s.e.m.) for five separate (five autopsies) determinations are presented for each tissue. Appropriate dilutions to perform gastrin radioimmunoassay on samples with an immunoreactivity below 80 pg gastrin per 50-μl or 200-μl sample

Table 2 Distribution of gastrin immunoassayable substance in nervous system of human and animals

Human	Dry weight	Gastrin assay	Animal	Dry weight	Gastrin assay
Central nervous system					
Cortical			Vertebrates		
grey matter	317±1	199±7	Mammals: Dog		
Cortical			Hemisph. brain	372±2	169±6
white matter	372±4	3±0.25	Cerebellum	360±2	<0.7
Lenticular			Birds: Pigeon		
nucleus	339±6	26±8	Hemisph. brain	338±4	130±7
Cerebral lobes	344±3	130±7	Cerebellum	351±5	<0.7
Hypothalamus	327±4	6±0.6	Fish: Trout		
Brain stem	360±4	8±0.6	Hemisph. brain	348±4	130±6
Cerebellum	330±2	<0.7	Amphibian: Frog		
Spinal cord	420±5	4±0.7	Hemisph. brain	340±4	125±5
Peripheral nervous system			Invertebrates		
Spinal ganglia	366±5	<0.7	Molluscan— <i>Loligo</i>	—	<0.5*
Sympathetic			Cerebroid		
ganglia	355±7	<0.7	Ganglia		
Peripheral roots	295±6	<0.7	Crustacean— <i>Homarus</i>	—	<0.5*
Peripheral			Ventral		
nerves	390±6	<0.7	Ganglia		

Tissue dry weight in mg per g wet weight, boiled extract gastrin immunoassayable material in ng per g dry weight. Means and standard errors of the mean (\pm s.e.m.) for three separate (three autopsies or three animals) determinations are presented. Appropriate dilutions to perform gastrin radioimmunoassay (gastrin assay) on samples with immunoreactivity below 80 pg gastrin per 50- μ l or 200- μ l sample.

* ng per g net weight.

2-17 previously reported²⁰. Disappearance of immunoreactivity of BGP after action of proteolytic enzymes, resistance of BGP to boiling for 15 min at 100 °C and its absorption on charcoal favour a small peptide molecule. Preliminary results with Sephadex G25 (columns 2.8 cm in diameter and 45 cm long) show that BGP is eluted between Gastrin 2-17 and ¹³¹I. This sets the molecular weight of BGP to less than that of Gastrin 2-17 (2,020).

BGP is different from releasing factors in that its highest concentration is found in the cortex. Of other natural substances related to gastrin by amino acid composition, cholecystokinin-pancreozymin and caerulein²¹, none has been described previously in cortical grey matter. The discovery in the vertebrate CNS of a new peptide reacting with gastrin antibodies is not unexpected in view of the postulated neural crest origin of the endocrine polypeptide (APUD) cells of the

gastrointestinal tract and pancreas²². The interest in brain-restricted peptides is that they are likely candidates for involvement in the function of the nervous system. At a time when the brain is increasingly being considered not only as a tissue with synaptic connections but also as an "endocrine organ and hormone target"²³, such a new peptide takes on an even greater significance.

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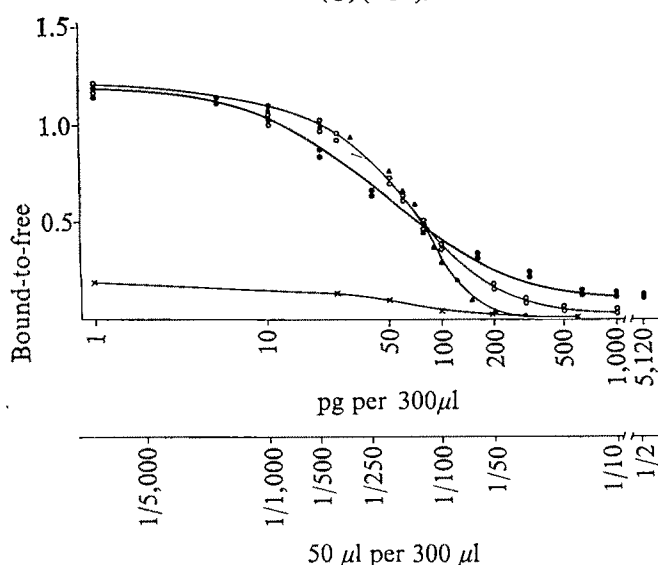
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Fig. 1 Radioimmunoassay for Gastrin 2-17. Bound-to-free ratio of labelled Gastrin 2-17 plotted against log dose of pg gastrin activity of solution of Gastrin 2-17 in a buffer of potassium phosphate (0.01 M), sodium chloride (0.15 M) at pH 7.4 with 10 mg ml⁻¹ egg albumin (○) in boiled brain extract from which immunoreactivity has (Δ) or has not (×) been removed by charcoal and of various dilutions of concentrated boiled brain extracts (●) (BGP).



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Role of sodium in neuronal uptake of monoamines and amino acid precursors

It is generally accepted that the neuronal re-uptake of monoaminergic neurotransmitters after their release into the synaptic cleft, inactivates transmitter action^{1,2}. The amino acid precursors L-tyrosine and L-tryptophan are also taken up by neurones from the circulation³⁻⁵. Using *in vitro* models of these processes, it has been demonstrated that tricyclic antidepressant drugs inhibit the uptake of both the amines^{6,7} and their precursors⁸ into synaptosomal fractions obtained from rat brain homogenates. Amino acids can be transported into cells by an Na⁺-dependent, Na⁺-independent or an Na⁺-inhibited transport system⁹. Monoamines, however, have been reported^{10,11} to have only an Na⁺-dependent system. Since information is lacking concerning the uptake of L-tyrosine and L-tryptophan, we have attempted to determine the role of Na⁺ ions in the uptake of the amino acid precursors compared with that of monoamines.

The uptake of the amino acids ¹⁴C-L-tyrosine and ¹⁴C-L-tryptophan and their related monoamines ¹⁴C-1-noradrenaline (NA), ¹⁴C-dopamine (DA) and ¹⁴C-5-hydroxytryptamine (5-HT) was studied in a crude synaptosomal fraction obtained from rat brain homogenate. The preparation of synaptosomal fractions and the methods used for the uptake experiments have been described⁸ (see legend to Fig. 1 for details). After incubation of the amino acids the pellet did not contain measurable amounts of formed monoamines (unpublished results and ref. 12). No attempt was made to separate the ¹⁴C-monoamines from metabolites formed, but, to prevent degradation of the monoamines by monoamine oxidase (MAO) an MAO-inhibitor (nialamide) was added to the incubation fluid⁶. The samples were centrifuged after incubation for 30 min at 70,000g, the supernatant discarded and the pellet rinsed twice with cold buffer. The pellet was dissolved in 1.0 ml deionised water by ultrasonic disintegration (5 s) and then 12 ml Instagel was added. Radioactivity was measured with a Packard liquid scintillation spectrometer.

Figure 1 shows the uptake of both amino acids by synaptosomes incubated in Krebs-phosphate buffer containing different Na⁺ concentrations and it can be seen that replacement of Na⁺ by sucrose resulted in an increased uptake. Thus, increasing the Na⁺ concentration inhibits the uptake of amino acid, but the uptake of the three monoamines studied is enhanced when the Na⁺ concentration is increased (Fig. 1b). Although it is known that the uptake of NA and 5-HT is Na⁺-dependent^{10,11}, a small amount is taken up in the absence of Na⁺. An effect of sucrose on the uptake processes seems rather unlikely since replacement by sodium by choline produced the same effects as replacement by sucrose (unpublished). Our results show that uptake of 5-HT was maximal at 50 mM Na⁺, whereas the uptake of NA and DA was maximal at about 100 mM Na⁺. *In vitro* conditions can, therefore, be created in which the uptake of 5-HT will be nearly maximal (25 mM Na⁺) without affecting the uptake of NA or DA. Optimal conditions for the uptake of NA, DA and 5-HT differ considerably from those for tyrosine and tryptophan.

Transformation of these results into a physiological model suggests that the re-uptake of monoamines and the uptake of amino acid precursors into nerve terminals is regulated by cation fluxes which are also responsible for the generation and conduction of impulses. During depolarisation, Na⁺ influx is increased and so an Na⁺ deficiency near the extracellular site of the axonal membrane may ensue. This Na⁺ deficiency will promote the uptake of tyrosine into the nerve ending, while at the same time NA or DA will be released into the synaptic cleft as a consequence of an increased influx of Ca²⁺ ions during depolarisation¹³⁻¹⁵. Both the increased intraneuronal tyrosine concentration and the release of NA

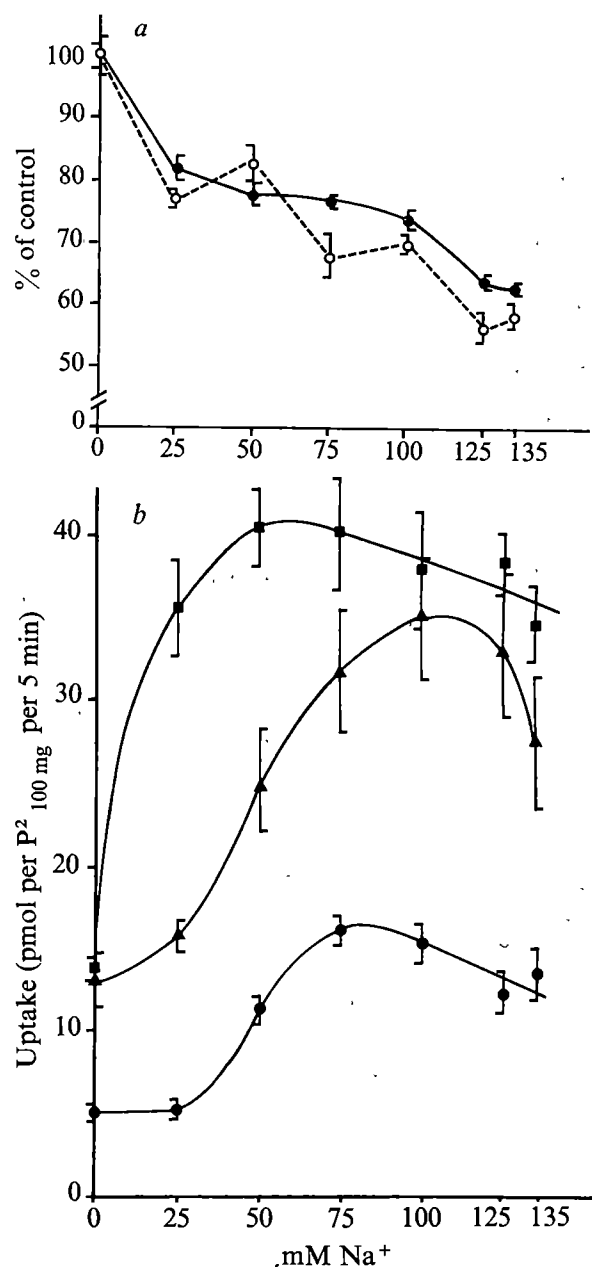


Fig. 1 *a*, Effect of Na⁺ concentration on the uptake of L-¹⁴C-tyrosine and L-¹⁴C-tryptophan by a synaptosomal fraction *in vitro*. The synaptosomal fraction was suspended in a Krebs-phosphate buffer (pH 7.4) (5 ml per g of tissue) containing (in mM): NaCl 118.46; KCl, 4.74; CaCl₂, 1.27; KH₂PO₄, 1.18; MgSO₄, 1.18; Tris-HCl, 16.15 and 40.0. For each incubation experiment 0.5 ml of the suspension, equivalent to a synaptosomal fraction from 100 mg of brain tissue, was used. Synaptosomes were preincubated for 15 min at 37 °C in a shaking incubator, and the ¹⁴C-labelled compound, dissolved in 0.5 ml Krebs buffer, was added and incubation continued for a further 5 min. Incubation was stopped by placing the incubation vessels in ice water. Buffers containing different Na⁺ concentrations were prepared by replacing NaCl isosmotically by sucrose. Uptake was measured 5 min after addition of L-¹⁴C-tyrosine (2×10^{-6} M; 3.4 mCi mmol⁻¹) or L-¹⁴C-tryptophan (0.25×10^{-6} M; 13.9 mCi mmol⁻¹). Quenching was measured by the automatic external standardisation (AES) ratio method. *b*, Effect of Na⁺ concentration on uptake of NA, DA and 5-HT by a synaptosomal fraction *in vitro*. Conditions were as described in *a* except that the incubation fluid also contained nialamide (10^{-4} M). Uptake was measured at bath concentrations of 5×10^{-6} M. Specific activities: NA, 54 mCi mmol⁻¹; DA, 57.3 mCi mmol⁻¹; 5-HT, 54 mCi mmol⁻¹. Compounds used and specific activities: L-tryptophan (methylene-¹⁴C), 52 mCi mmol⁻¹; L-tyrosine-¹⁴C (U), 522 mCi mmol⁻¹; 5-HT-3'-¹⁴C creatinine sulphate, 55 mCi mmol⁻¹; dopamine (ethylamine-2-¹⁴C) hydrochloride, 57 mCi mmol⁻¹; 1-noradrenaline *d*-bitartrate (methylene-¹⁴C), 57 mCi mmol⁻¹ (all Radiochemical Centre, Amersham). *a*, ●, L-tyrosine; ○, L-tryptophan; *b*, ●, NA; ▲, DA; ■, 5-HT.

or DA will enhance biosynthesis of these transmitters, because more precursor is now available and the product feedback repression of tyrosine hydroxylase is removed¹⁶⁻¹⁸. Furthermore, the biosynthesis of NA or DA may be increased by the influx of both cations which have been reported to activate tyrosine hydroxylase^{18,20}.

During the restoration phase Na⁺ ions are expelled into the extracellular space which induces re-uptake of monoamines. During the resting state the uptake of monoamines is thus optimal, while the synthesis and release of monoamines is inhibited. During depolarisation, however, the release and synthesis of monoamines is activated and at the same time the re-uptake of tyrosine and tryptophan is enhanced. During this phase of the action potential the re-uptake of monoamines is inhibited. This impairment of the uptake of monoamines, after their release into the synaptic cleft, potentiates the action of the released transmitter on its receptor. The same mechanism for a coupling between impulse inflow or cation fluxes and release, synthesis and re-uptake of transmitter can be applied to 5-hydroxytryptaminergic nerves, but in this case the uptake of tryptophan may be more critical for an increased biosynthesis of 5-HT since as yet no product feedback repression of tryptophan hydroxylase is known.

Since the most important assumption of this hypothesis is the extracellular deficiency of Na⁺ ions near the axonal membrane during depolarisation, a theoretical calculation concerning this problem is given below. If one assumes that the area of the presynaptic membrane is 1 μm^2 and the extracellular Na⁺ ion concentration is 0.1 M, the number of Na⁺ ions per μm^3 will be $10^{-16} \times 0.1 \times \text{Avogadro's number } (N)$. N is 6×10^{23} , so the number of Na⁺ ions per μm^3 will be 6×10^7 . Assuming a uniform distribution of Na⁺ ions, the number of Na⁺ ions occupying 1 μm^2 will be $(6 \times 10^7)^{2/3} = 15 \times 10^4$ Na⁺ ions. In non-myelinated nerve tissue each impulse will cause an influx of 20,000 Na⁺ ions per μm^2 (ref. 19), so deficiency near the axonal membrane might occur if the diffusion of extracellular Na⁺ is slow enough.

In cases of frequent stimulation, it is not unlikely that after seven impulses a complete layer of Na⁺ ions will have disappeared. In the synaptic cleft, which has a volume of about 0.02 μm^3 , only eight layers of Na⁺ ions are present, each consisting of 150,000 Na⁺ ions. These ions not only serve to conduct the presynaptic action potential, but may also be needed for the generation of a postsynaptic action potential. Although no data are available of the diffusion of Na⁺, or other ions, near the axonal membrane or in the synaptic cleft, one might expect that in a supercapillary system such as the synaptic cleft, the pre- and postsynaptic resting potential would exert a strong influence on the displacement of ions. The negative resting potentials at the pre- and postsynaptic

site will fix the position of Na⁺ ions in the synaptic cleft. During depolarisation of the presynaptic membrane, 20,000 Na⁺ ions will enter the presynaptic nerve ending, resulting in a decrease of the negative potential. Therefore, during depolarisation, the remaining Na⁺ ions present in the synaptic cleft will shift to the postsynaptic site, creating an Na⁺ deficiency near the surface of the presynaptic membrane. Thus, supposing that during depolarisation the displacement of extracellular Na⁺ ions is sufficiently slow, an Na⁺ deficiency near the axonal membrane might be expected. This condition will be favourable for an increased uptake of amino acid precursors simultaneously with an inhibition of the re-uptake of the stimulus-induced released monoamines. During the restoration phase, when the efflux of Na⁺ ions is increased, the re-uptake of monoamines is restored, and the biosynthesis will slow down as a result of product feedback repression and (or) an impairment of the uptake of amino acid precursor (Fig. 2). In this way synthesis, release and re-uptake of monoaminergic neurotransmitters are all coupled to cation fluxes which occur during nervous impulse flow.

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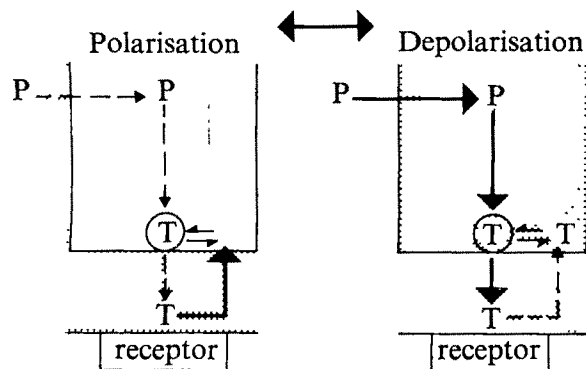
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Fig. 2 Hypothesis for the effect of impulse flow on the re-uptake, synthesis and release of monoaminergic neurotransmitters. P, Precursor; T, transmitter; ---, low activity or inhibition; —, increased activity.



Na⁺ and Ca²⁺ ions

Enteropancreatic circulation of digestive enzyme as a conservation mechanism

THE permeability of the intestine to pancreatic digestive enzymes has been known for some years¹⁻⁷. Recently it was demonstrated⁸ that chymotrypsinogen and amylase are transported across the pancreatic acinar cell as well. Taken together these observations suggest that some of the digestive enzymes which are secreted into the intestinal lumen are circulated by way of the bloodstream and acinar cell back into the intestine. This prediction was borne out experimentally. ³H-chymotrypsinogen instilled into the intestinal lumen reappeared in pancreatic secretion within minutes⁸. The question remained, however, whether this "enteropancreatic circulation" was a trace process either of a trivial nature or serving a regulatory function; or, whether—analogueous to the enterohepatic circulation of bile salts—it was a process in which substantial amounts of digestive enzyme could be circulated and thereby conserved. The

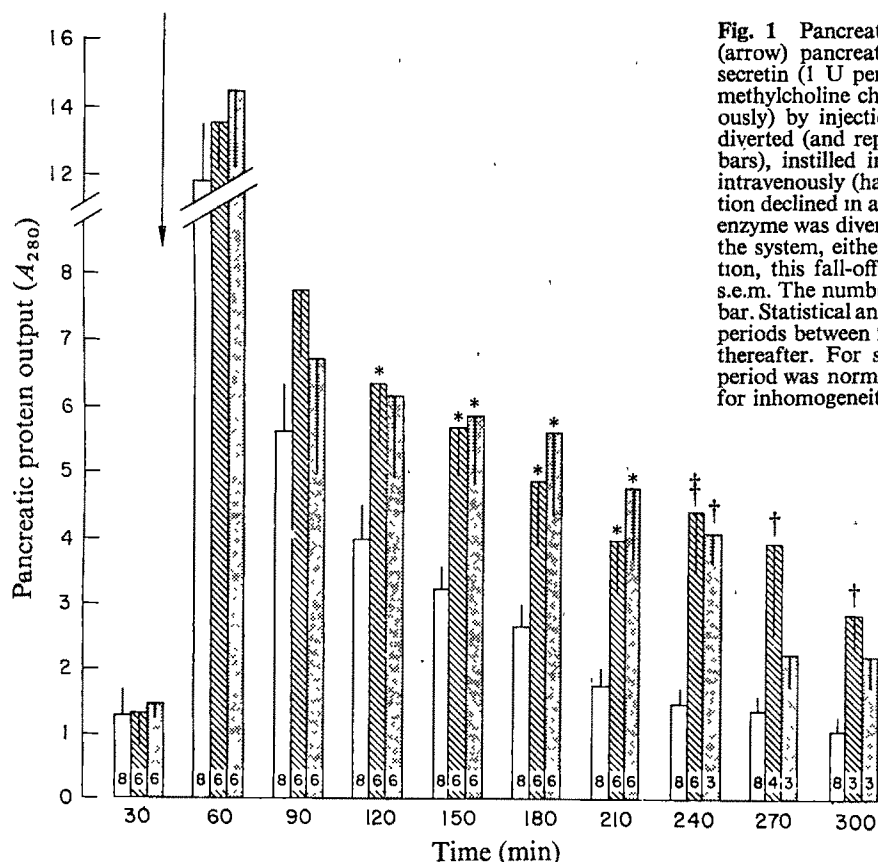


Fig. 1 Pancreatic protein secretion with time. After 30 min (arrow) pancreatic secretion was continuously stimulated with secretin (1 U per kg body weight, intravenously) and acetyl- β -methylcholine chloride (0.5 mg per kg body weight, subcutaneously) by injections every 30 min. Pancreatic juice was either diverted (and replaced by an equal quantity of albumin) (open bars), instilled into the duodenum (stippled bars), or injected intravenously (hatched bars). In these conditions, enzyme secretion declined in a roughly exponential manner when the digestive enzyme was diverted (control). When enzyme was added back to the system, either by intravenous injection or intestinal instillation, this fall-off was less pronounced. Error bars indicate the s.e.m. The number of experiments is given at the bottom of each bar. Statistical analysis was done using the Mann-Whitney test for periods between 90 and 150 min and Student's *t*-test for periods thereafter. For statistical analysis the protein output of each period was normalised for the peak response (60 min) to control for inhomogeneity of variance between experiments. **P* < 0.05; †*P* < 0.025; ‡*P* < 0.01.

experiments reported here are consistent with this latter view and suggest the circulation of approximately 60% of a mixture of digestive enzymes instilled into the duodenum (82–92% at steady state).

The pancreatic duct of male white New Zealand rabbits weighing 2–3 kg was cannulated *in situ* after a median laparotomy under DIAL-urethane anaesthesia (0.7 ml per kg body weight intraperitoneally) and subsequent to a 20 h fast. The continuity of the gastrointestinal lumen was maintained. Protein content was estimated by ultraviolet absorption at 280 nm. These measurements were comparable with values obtained with the Folin phenol reagent (protein content was estimated using both methods in selected experiments)⁹. After a 30-min unstimulated collection period, maximal secretion of both protein and fluid was continuously elicited by half-hourly injections of acetyl- β -methylcholine chloride (0.5 mg per kg body weight subcutaneously) and porcine secretin (1 Ivy dog unit per kg body weight intravenously) (kindly provided by Professor V. Mutt of the Karolinska Institutet). Samples were collected at 30-min intervals.

When pancreatic juice was completely diverted from the intestine, a roughly exponential decay in protein secretion was seen. In the continued presence of the cholinergic stimulus, secretion decreased by about 90% over a 4.5-h period (Fig. 1), although it was still enhanced relative to the secretion of protein from unstimulated glands at the same time (4.5 h).

While this decay or fall-off in protein secretion might have resulted solely from fatigue in the system, for example

decreased responsiveness to the stimulus or energy limitations relative to transport, it might also have been due to the diversion of digestive enzyme itself, which would remove the supply of available enzyme from a presumptive enteropancreatic circulation. To distinguish between these two possibilities, we collected pancreatic digestive enzyme secretion, stimulated as described above, measured its protein content, and immediately reintroduced it into the duodenum (without any modification of the material), thereby maintaining the continuity of the natural process. Under these conditions the fall-off was indeed less than during diversion (Fig. 1) (log protein output against time for the data in Figure 1; $b_{\text{diverted}} = -0.004$ ($r = 0.97$), $b_{\text{instilled}} = -0.0028$ ($r = 0.94$); b_{div} against b_{inst} , $P < 0.025$). A relatively steady protein output was maintained under these conditions for about 3 h, although not at maximal rates. The increase in protein secretion after protein instillation into the intestinal lumen was equal to 61.3 ± 10.6 s.e.m. ($n = 6$) of the amount instilled for the complete experiment (relative to time-paired controls (diverted)), and reached a maximum of 83% for the period of peak response (Table 1). This increase in pancreatic protein output might have come about indirectly from the release of hormones into the blood after the instillation of pancreatic juice into the intestine as well as from the direct circulation of enzyme molecules. To test for this possibility, at least for the hormone known to produce substantial changes in total protein output by this gland, cholecystokinin-pancreozymin (CCK-PZ), an experiment was done in which pancreatic juice was again completely diverted, but at 120 min 1 Ivy

Table 1 Circulation of pancreatic enzymes under steady-state conditions

Condition	<i>a</i> Protein input at 210 min	<i>b</i> Protein output 210–240 min	<i>c</i> Control output 210–240 min	<i>b</i> – <i>c</i>	Percentage recirculated, [(<i>b</i> – <i>c</i>)/ <i>a</i>] × 100
Intestinal instillation	3.1	4.1	1.5	2.6	83%
Intravenous injection	3.6	4.4	1.5	2.9	80%

dog unit of purified CCK-PZ per kg body weight was injected in addition to the other stimulants (as in Fig. 1) for the remainder of the experiment. No significant additional augmentation of protein secretion was seen under these conditions.

By contrast, if the circulation of intact digestive enzyme did indeed account for the increased protein output, then the intravenous injection of pancreatic juice should produce a similar response to that seen after intestinal instillation. This was observed (Fig. 1). There was an increase in protein secretion relative to controls equal to $51.6\% \pm 5.5$ s.e.m. of the protein injected during the course of the complete experiment with a maximum of 80.2% for the period of peak response (Table 1) (log protein output against time for the data in Fig. 1; $b_{\text{injected}} = -0.0022$ ($r = 0.94$); b_{div} against b_{inj} , $P < 0.001$).

The relationship between the amount of digestive enzyme instilled or injected and increased enzyme secretion as a result of either can be more clearly evaluated by plotting the response as a function of the amount of digestive enzyme instilled or injected in individual experiments. This is true because the amount of enzyme applied varied considerably from experiment to experiment. When the data were treated in this manner, the variance virtually disappeared and the response was proportional in a roughly linear fashion to input ($y = 0.92x + 3.75$, $r = 0.97$). The calculated slope was now 0.92 or the equivalent of 92% circulation. The difference between the overall responses (61% for instillation and 52% for injection) and the slope for the data as presented in Fig. 2 is found in the positive x-intercept ($x = 19.7$), which represents the amount of enzyme that must be added to the system before there is a response. This is the system 'capacitance' and may include various enzyme-containing compartments, such as the intestinal lumen, absorptive cells, blood, kidney, and of course the pancreas itself. Apparently, the size of the capacitance is roughly the same regardless of how the enzyme is administered since both injected and instilled experiments regressed similarly with approximately equal intercepts ($x_{\text{inst}} = 18$, $x_{\text{inj}} = 21.5$). The fact that one point was negative suggests that our function as calculated is slightly

displaced. If we correct this by setting the difference between control and experimental values equal to zero for the lowest response (the negative point) and adjust the other experimental points equivalently, the positive intercept of the x-axis is still substantial (uncorrected x-intercept = 19.7, corrected x-intercept = 13.5) and significantly different from 0 ($P < 0.001$).

While circulation of digestive enzyme is the most direct interpretation of these data, particularly since the potential for such a process has been demonstrated⁸ and also because input and output are almost equivalent at the steady state, the injected or instilled enzyme could still have produced the effect in other ways such as by the release of as yet undescribed secretagogues or by regulating enzyme secretion directly at the acinar cell level. If indeed the enzyme is being circulated, then, at least for the situation of maximally stimulated secretion in the absence of food, the efficiency of the process is remarkably high, of the order of 80–90%.

The conservation of digestive enzyme by its circulation could provide for a substantial saving of energy for digestion, that is, it would eliminate at least in part the need to metabolically recycle digestive enzyme by the liberation (protein breakdown), absorption, and reincorporation of amino acids into new protein.

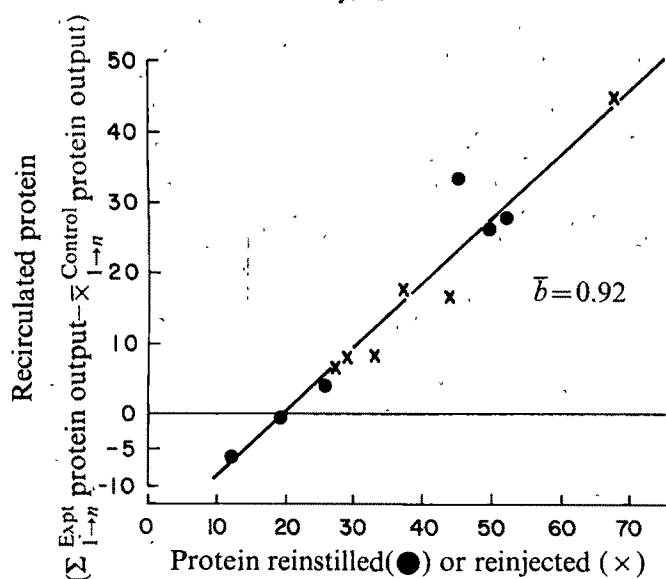
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Fig. 2 The relationship between enzyme input (intestinal instillation (●) or intravenous injection (×)) and the circulation of digestive enzyme in each individual experiment (the difference between total protein output in each experiment ($\Sigma_{1 \rightarrow n}^{\text{Expt}}$) and the average total output for control experiments for the same duration ($\bar{X}_{1 \rightarrow n}^{\text{Control}}$). The amount of enzyme circulated varied directly and linearly with the amount of enzyme added to the system with a slope of 0.92 or 92% of the input being circulated. The positive x-intercept represents the input capacitance of the system.



Increased breakdown of glycosaminoglycans and appearance of corrective enzyme after skin transplants in Hunter syndrome

ATTEMPTS to treat the mucopolysaccharidoses with infusions of normal plasma or leukocytes have produced mixed results; some workers^{1–3} reported little or no positive effect, while others^{4–6} noted both clinical and biochemical changes after treatment. Among the more obvious reasons for these conflicting findings are differences in the responsiveness to treatment of genetically distinct forms of the disorders⁷, in the volumes of plasma administered and in the activities of the corrective factors in the plasma.

Replacement therapy by infusion of plasma can at best provide only a temporary solution, since the half lives of the enzymes involved are of the order of a few days only^{8–10}. The changes we observed immediately after infusion^{6,7,11,12} were, however, sufficient to encourage an attempt to provide a more permanent source of corrective factors. Accordingly, skin allografts were transplanted on a 4-yr-old boy (B.G.) with Hunter syndrome, who, 11 months earlier, had shown a positive response to plasma infusion therapy¹². The diagnosis was established first on the typical clinical features, second on probable X-linked inheritance, since the mother had another affected son by an earlier marriage and third, by cultured fibroblast correction experiments using typed reference cells. Both the mother (HL-A:

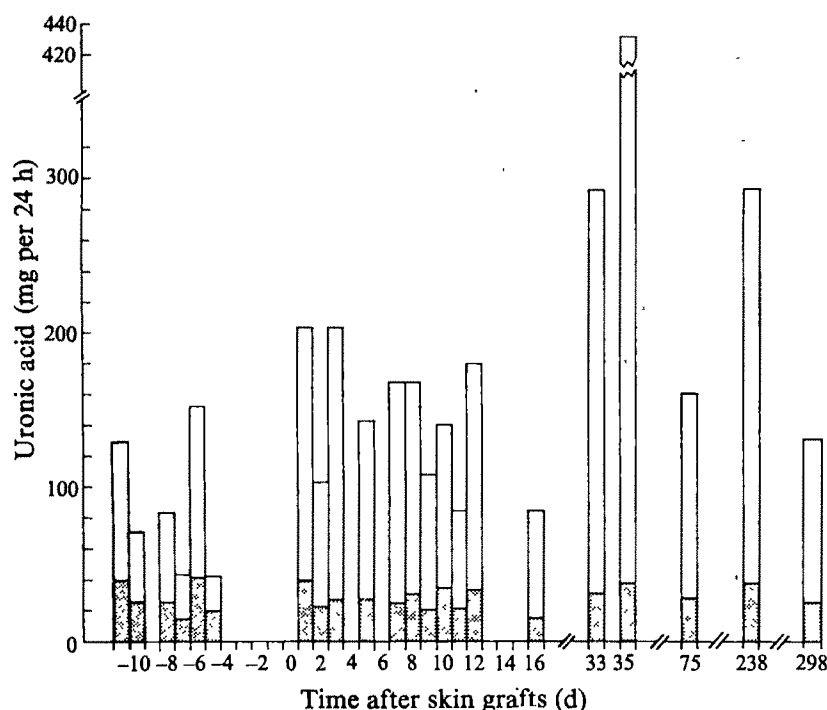


Fig. 1 Total 24-h excretion of urinary uronic acid before and after skin grafting. The day of grafting is designated day 0, and the days before grafting by negative numbers. Stippled areas of columns, polymeric uronic acid; open areas of columns, uronic acid fragments

W32,9,12,y) and the father (HL-A:1,2,8,17) had two HL-A antigens in common and two incompatible with the patient (HL-A: 1,9,8,y) (y indicates an unidentified antigen of the second series). In the mixed leukocyte reaction the patient's cells had no reaction when mixed with maternal cells but showed an approximate threefold increase in incorporated radioactivity when mixed with paternal leukocytes. But the patient received skin allografts from both parents, because it was reasoned that the dose of genes responsible for the Hunter corrective factor might be higher in skin cells derived from the father than from the mother (who was an obligatory heterozygote in whom an unknown proportion of wild genes on Lyonised X chromosomes might be inactive).

Full thickness elliptical skin 5 cm × 2 cm was taken from the inner side of the left upper arm of the mother and father, and grafted on the flexor aspect of the forearm of the child. Immunosuppressive treatment (azathioprine, 25 mg daily, prednisolone, 25 mg twice daily), was commenced 7 d before grafting and continued (at the same doses for 6 weeks and then on progressively smaller doses) for 9 months.

Initially both grafts were judged to have taken and were pink after 2 weeks. Four weeks after transplant the paternal graft was rejected. Six weeks after transplant it was judged that about 90% of the maternal graft had taken, but this too was rejected about 3 months after transplantation.

Urine collections (24 h) taken before and after grafting were stored at -20 °C with 10 ml of toluene until analysed. Polymeric glycosaminoglycans (GAG) were isolated from 200-ml samples by precipitation with 9-aminoacridine HCl and conversion to their sodium salts^{13,14} and the GAG fragments that remained in the supernatant solution were collected on a column of Dowex-1 × 2 (ref. 4) and eluted with 2 M sodium chloride. Both fractions were assayed for their uronic acid contents¹⁵. The GAG fragments eluted from Dowex were concentrated by positive pressure dialysis through a filter membrane (UM-05, Amicon) and 1-ml samples containing approximately 1 mg of uronic acid were chromatographed on a column (13 mm × 600 mm) of Sephadex G-25 'fine'. The relative proportions of high and low molecular weight fractions I and II respectively (Fig. 2) were determined by tracing the elution profiles on to thin paper, then cutting out and weighing the area under

each peak. Sulphate-uronic acid ratios were determined as before¹¹.

Crude Hunter corrective factor was isolated from urine samples using part of the method described by Cantz *et al.*⁸. The precipitate from urine made 70% saturated with ammonium sulphate was redissolved in water, insoluble residue was discarded after centrifugation, and the supernatant solution was made 50% saturated with ammonium sulphate. The resulting precipitate was removed by centrifugation and the supernatant solution was made 80% saturated. This last precipitate was again dissolved in water, freeze-dried and stored at -15 °C. Weighed amounts were redissolved in 1 ml of 0.01 M phosphate buffer, pH 6.0, in 0.15 M NaCl, dialysed against three changes of the same buffer (500 ml) at 2 °C for 20 h, then assayed for corrective activity⁸ using cultured fibroblasts from the patient.

Some 25–30% of the patient's total 24-h uronic acid was excreted as polymeric GAG before grafting (Fig. 1). After treatment the proportion of polymeric GAG decreased considerably, varying between 15 and 20% while at the same time total uronic acid excretion increased markedly, reaching a peak almost three times higher than pretreatment levels, on the 35th day after grafting. At this time polymeric GAG accounted for less than 10% of the total.

The GAG fragments eluted from Dowex were separated

Table 1 Changes in the size distribution and sulphate-uronic acid ratio of uronic acid oligosaccharides eluted from Sephadex G-25 before and after skin grafts

Day of treatment	Percentage of total uronic acid eluted		Sulphate-uronic acid ratio of Fraction II
	Fraction I	Fraction II	
- 12	48	52	1.7
- 9	48	52	—
- 5	56	44	—
+ 5	33	67	2.1
+ 11	40	60	—
+ 15	33	67	—
+ 33	26	74	—
+ 235	31	69	1.1
+ 296	16	84	1.2
+ 422	14	86	0.5

Fractions I and II are the high and low molecular weight components respectively shown in Fig. 2.

into three components by chromatography on Sephadex G-25 (Fig. 2), of which the largest was eluted closely behind the void volume followed by an oligosaccharide component of smaller molecular size and finally a tail fraction. Before treatment, the largest of these components contained approximately half the total uronic acid eluted from the column (Table 1), but by the fifth day after grafting this had decreased to one third of the total and the proportion continued to decrease with time, amounting to 26% by day 33 and 14% by day 422. Coupled with the increase in relative proportions of component II was a decrease in the ratio of SO_4 :uronic acid of this fraction from values near 2.0:1 down to only 0.5:1, 422 d after grafting.

Three normal children aged 4.5–8 yr excreted 89.2–104.7 U of Hunter corrective factor in their urine every 24 h. (1 U was defined by Cantz *et al.*⁸ as sufficient to induce half maximal correction of sulphate incorporation.) In contrast, the patient 270 d before skin transplant (and 42 d after receiving 1,600 ml of plasma from normal blood)

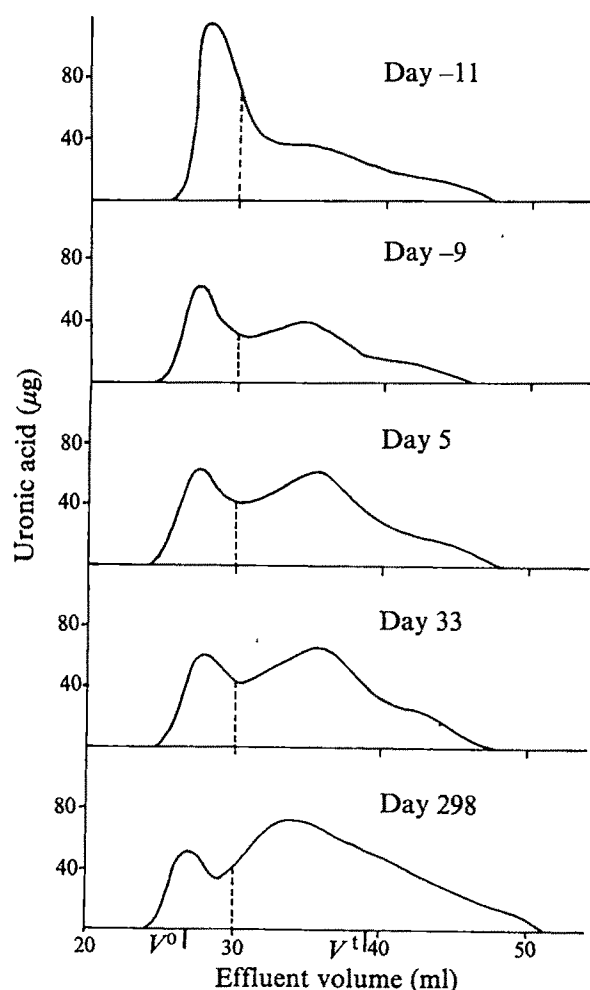


Fig. 2 Sephadex G-25 gel filtration profiles of uronic acid fragments eluted from Dowex-1. Samples containing 1 mg of uronic acid were eluted from the column in 0.2 M sodium acetate, pH 6.8. Fractions (0.85 ml) were collected and their uronic acid contents determined by an automated procedure. The dotted line indicates the boundary between fractions I and II (see Table 1). Sephadex filtration analysis was carried out on each of the six preoperative samples represented in Fig. 1. The two which are shown were selected because they show the extremes of preoperative variation. Thus the profile on day -9 had the greatest oligosaccharide component of any of the preoperative samples.

Table 2 24 h Urinary excretion of Hunter corrective factor

Source of urine	*Units of correction excreted per 24 h
Female control, age 4 yr 6 months	89.2
Male control, age 8 yr	104.7
Male control, age 4 yr 6 months	95.2
Patient, day -270†	0.86‡
Patient, day 15	0.00
Patient, day 54	0.12
Patient, day 98	3.62
Patient, day 237	4.89

*Units of correction were calculated from a dose-response curve constructed by plotting the degree of correction of the patient's fibroblasts (fall in radiol sulphate incorporation per mg of fibroblast protein) induced by serial dilutions of corrective factor⁸. Duplicate values agreed within $\pm 2.4\%$ except those for the female control which agreed to within $\pm 6.1\%$. Samples prepared from control urines and from the patient gave a mean corrective value of 0.34 units per 24 h after they had been boiled for 5 min. This control level was subtracted from all results obtained with unboiled preparations.

†Day 0 is defined as the day on which skin transplant was performed.

‡This urinary collection started 42 d after the patient had received an intravenous infusion of 1,600 ml of plasma from normal blood.

excreted only 0.86 U. As Table 2 shows, corrective factor excretion was low until 54 d after transplant but had risen significantly 98 and 237 d after transplant.

The large increase in excretion of uronic acid and in the relative proportions of oligosaccharides of low molecular weight, strongly suggest that in this patient, the grafts had released sufficient Hunter corrective factor to induce these changes. Since the defective enzyme in the Hunter syndrome is sulpho-L-iduronate sulphatase¹⁰, the increased desulphation of low molecular weight GAG further supports this view. We have reported similar evidence for GAG degradation after infusion of normal plasma^{6,7,11,12}, but on this occasion nonspecific effects attributable to an increase in blood volume after transfusion could be discounted; moreover the production of the Hunter corrective factor was confirmed by demonstrating its appearance in the urine.

The amount of corrective factor excreted increased during the period of observation, even though both grafts seemed clinically to have been rejected by day 100. It is possible, however, that some of the donor cells survived longer, since the amount of corrective factor excreted was still greatly in excess of pretreatment levels 237 d after grafting (Table 2), although its half life *in vitro* is only 2 d (ref. 8). Active concentration by fibroblasts of deficient enzymes has previously been demonstrated *in vitro* for other types of mucopolysaccharidosis^{9,10}. Thus, the delayed excretion of Hunter corrective factor observed in this case can be explained if all of the enzyme produced by the graft initially was taken up by the patient's cells. Alternatively the data are also consistent with the explanation that cells in the graft produced a factor which stimulated the patient's cells to produce corrective factor.

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Human serum lyses RNA tumour viruses

ALTHOUGH there is agreement that oncornaviruses infect and are associated with malignancies in mice, cats and chickens¹, the evidence for oncornavirus infection in man remains controversial². Similarly, antibodies to oncornavirus antigens have been detected in laboratory animals³⁻⁷, but there are few data indicating the presence of such antibodies in humans^{8,9}. These findings suggest that man possesses a natural defence mechanism which inhibits or interferes with oncornavirus infection and replication. We report here evidence for such a mechanism.

Oncornaviruses isolated from several animal species were found to be inactivated by fresh human serum. As determined by an XC cell plaque reduction assay¹⁰, a 1:2 dilution of human serum inactivated 2.5×10^5 plaque-forming units (PFU) of Moloney leukaemia virus (MLV). The 50% end-point dilution for all of five human sera tested was approximately 1:16 for AKR leukaemia virus, Friend leukaemia virus, MLV, and Rauscher leukaemia virus (Table 1). Similarly, by using a focus reduction assay, a 1:16 dilution of human serum inactivated Moloney sarcoma virus (MSV), simian sarcoma virus, and MSV-gibbon ape leukaemia virus pseudotype. There was, however, no demonstrable inactivation of oncornaviruses by heated (56 °C, 30 min) human serum or by fresh sera from guinea pigs, rabbits, or mice (BALB/c and NIH Swiss). To determine the mechanisms of viral inactivation, SCRF 179 cells, an established murine lymphoid line free of mycoplasma and persistently infected with MLV (SCRF 179 (MLV)), were grown in medium con-

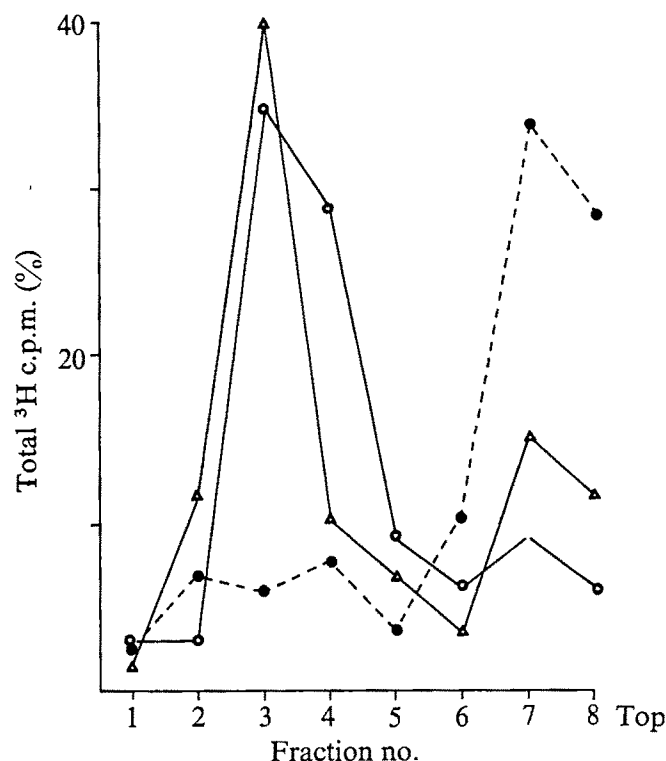


Fig. 1 Effect of human serum on sedimentation of MLV. MLV, labelled with ³H-uridine, was purified in sucrose gradients and, without a freeze-thaw, was reacted at 37 °C for 30 min with fresh human serum (●), heated (56 °C, 30 min) human serum (○), or 1% bovine serum albumin (△). The mixtures were layered on to 4.5 ml 20 to 50% (w/w) sucrose gradients in TES buffer (0.01 M Tris, pH 7.4; 0.05 M NaCl; 0.001 M EDTA). The gradients were centrifuged in a Spinco rotor SW 50.1 at 50,000 r.p.m. for 1 h and fractionated into 0.7-ml samples. Counts were made in Aquasol (New England Nuclear) using 20-μl samples.

taining ³H-uridine (15 μCi ml⁻¹; 40-50 Ci mmol⁻¹). Labelled MLV was purified, incubated for 30 min at 37 °C with bovine serum albumin (BSA), heated human serum or fresh human serum, and analysed by sucrose gradient ultracentrifugation. Figure 1 shows that after being incubated with either BSA or heated human serum the virus-associated radioactive counts sedimented to the lower portion of the gradient. This radioactivity was precipitable by 10% cold trichloroacetic acid (TCA) and resisted degradation by RNase (20 μg ml⁻¹). In contrast, when MLV was mixed with fresh human serum, most of the label remained at the top portion of the gradient and was not quantitatively precipitated by TCA. These results indicated that fresh human serum released RNA from the virus, presumably by lysis¹¹.

Fresh but not heated human serum released RNA-dependent DNA polymerase (RDDP) from six different oncornaviruses (Table 2) and such RDDP release could be

Table 1 Inactivation of oncornaviruses by human serum

Virus	Untreated	Plaque number after treatment:					With heated serum 1:2
		1:2	1:4	1:8	1:16	1:32	
AKR leukaemia virus	185	0	0	0	42	112	190
Friend leukaemia virus	206	0	0	0	48	127	210
Rauscher leukaemia virus	489	ND	0	14	69	370	498
Moloney leukaemia virus	880*	4	18	49	139	620	811

Equal volumes of virus and human serum (fresh or heated at 56 °C for 30 min) dilutions were incubated at 37 °C for 30 min. Duplicate 0.4-ml samples of the virus-serum mixtures were inoculated on to monolayers of secondary NIH Swiss mouse fibroblasts (24 h old) in the presence of polybrene (2 μg ml⁻¹). Cultures were refed 1 and 4 d after infection, irradiated with ultraviolet light, and overlaid with 10⁶ XC cells per Petri dish¹⁰. These cells were refed 1 d and fixed and stained 3 d after irradiation.

*Represents 88 plaques of a 1/10 dilution of input virus multiplied by 10.

Table 2 Release of oncornavirus RDDP by human serum

Enzyme source	Virus source	Serum type	c.p.m. polymerised ³ H-TTP	
			Fresh serum	Heated serum
None-H ₂ O	—	—	327	376
Purified RDDP*	—	Normal	1,070	943
Avian myeloblastosis virus	Chicken serum	Normal	728	350
Feline leukaemia virus	NCI	Normal	6,280	1,169
Feline leukaemia virus	NCI	C4 depleted	849	842
Moloney leukaemia virus	YCAB cells	Normal	2,153	466
Moloney leukaemia virus	YCAB cells	C2 deficient	587	587
Moloney leukaemia virus	SCRF 179 cells	Normal	3,064	393
Moloney leukaemia virus	SCRF 179 cells	C4 depleted	495	474
Rauscher leukaemia virus	NCI	Normal	2,383	897
Rauscher leukaemia virus	NCI	C2 deficient	730	696
Simian sarcoma virus	NCI	Normal	708	452
Wild mouse virus 1504	Wild mouse 1504 embryo cells	Normal	1,055	343

Enzyme source (25 μ l) was incubated with 25 μ l fresh or heated (56 °C, 30 min) human serum for 30 min at 37 °C. After incubation an equal volume of RDDP assay mix was added to the RDDP-serum solution, and the samples were again incubated at 37 °C for 30 min. The assay mix contained 1.25 mM MnCl₂; 0.05 M Tris buffer, pH 8.2; 20 mM dithiothreitol; 100 μ M deoxyadenosine 5'-triphosphate, 100 μ M deoxycytidine 5'-triphosphate; 100 μ M 2-deoxyguanosine 5'-triphosphate; 10 μ M thymidine 5'-triphosphate; poly(rA)-oligo(dT)₁₂₋₁₈ (0.33 U ml⁻¹) and thymidine-methyl-³H 5'-triphosphate (100 μ Ci ml⁻¹, specific activity 18 Ci mmol⁻¹). Polymerised ³H-TTP was counted in Aquasol after collection on DEAE filters and subsequent washes with 5% Na₂HPO₄, water, ethanol and ether¹². The degree of RDDP release caused by serum (which influences the RDDP assay) compared with NP40 varied greatly with each virus preparation and depended on virus concentration and amount of inactivated virus. For instance, this preparation of feline leukaemia virus released 108,000 c.p.m. of enzyme activity after NP40 treatment, whereas the simian sarcoma virus preparation released only 8,200 c.p.m. after NP40 treatment.

*Purified RDDP was from MLV.

detected at serum dilutions up to 1:8. Since RDDP is located within the virion, the release of this enzyme is a measurement of viral lysis. All normal sera from 32 separate humans tested but none from six individual guinea pigs, four individual rabbits, or pools from two separate strains of mice (BALB/c, NIH Swiss) caused the release of RDDP. The tested normal human sera were from five different cord blood samples and from six leukaemic and 21 healthy adults of both sexes with functional complement (C) activity.

Table 2 also shows that human sera genetically deficient in C2 or immunochemically depleted of C4 failed to release RDDP. These observations, coupled with the finding that all tested fresh normal sera released RDDP, suggested that C was required for the viral lysis. Consistent with this interpretation is the finding of C activation by MLV. After mixture of 200 μ l of fresh human serum with 200 μ g of purified MLV (in 200 μ l) for 60 min at 37 °C, 89% of the total complement haemolytic 50% endpoint (CH₅₀) units were consumed. Comparable C consumption was also observed when the reaction of serum with MLV was carried out in the presence of RNase (2.3 mg ml⁻¹) and DNase (1.6 mg ml⁻¹) (final concentrations in the serum-virus mixture), indicating that viral or contaminating cellular RNA or DNA was not responsible for the observed consumption of C. Haemolytic C component titrations¹³ in serum incubated with virus revealed significant consumption of the terminal components of C, that is C8 (40% above background) and C9 (20% above background), which are required for membrane lysis in other systems^{13,14}. In addition, the conversion of C3 and C4 in the serum was demonstrated by immunoelectrophoresis.

We were unable to establish a role for antibody in this reaction after trying four methods. First, fluoresceinated monospecific antisera against human IgA, IgG or IgM failed to stain the surfaces of SCRF 179 (MLV) cells which had previously been incubated with human serum. These cells demonstrated cell surface MLV antigens detectable by immunofluorescence with rat antibodies to MLV. Second, human serum heated at 56 °C for 30 min failed to inhibit oncornavirus plaque formation or to release RDDP either alone or in conjunction with fresh guinea pig or rabbit sera. Third, sera from two agammaglobulinaemic patients released RDDP from oncornavirions. These sera had less than 10 μ g IgG per ml and no detectable IgA or IgM. Fourth, fresh human serum retained its ability to release RDDP from oncornaviruses (MLV and feline leukaemia

virus) after absorption with SCRF 179 (MLV) cells. (This serum was absorbed twice with 1×10^7 cells for 40 min at 4 °C in the presence of 0.01 M EDTA. Before use, the EDTA in the serum was neutralised with CaCl₂.)

To rule out the possibility that lysis of oncornaviruses occurred because of C activation by components of foetal calf serum retained on the viral surface, we purified MLV from SCRF 179 (MLV) cells grown in medium containing heat-inactivated human serum in place of foetal calf serum. When mixed 1:2 with fresh human serum, these virions released significant amounts of RDDP and did not form plaques on XC cells (control without serum-3180 PFU, heated serum-2880 PFU, fresh serum-0 PFU). Avian myeloblastosis virus purified directly from chicken serum also released RDDP on incubation with fresh human serum (Table 2), and MLV obtained directly from a 1:10 dilution of mouse plasma (14,500 PFU) was inactivated by a 1:2 dilution of fresh human serum (heated human serum—confluent (>5,000) PFU, fresh human serum—263 PFU). Fresh guinea pig serum did not reduce plaque formation by this same MLV preparation from mouse plasma.

Our results demonstrate that all tested human sera with functional C sources inactivate and lyse avian, feline, murine and simian oncornaviruses. Whether a similar mechanism inactivates other enveloped viruses is unknown and is under study in our laboratory. C seems to be a mediator of this lytic mechanism, since human sera deficient in C2 or C4 do not release RDDP from oncornaviruses. In contrast, we found no evidence for antibody involvement in this reaction. Not only did agammaglobulinaemic human sera lyse oncornaviruses, but absorption of normal human serum with cells expressing surface oncornavirus antigens also failed to remove its cytolytic potential, and heated human serum was unable to sensitise oncornaviruses for lysis with guinea pig C. These findings suggest either that trace amounts of heat-labile antibody are present or that human C but not guinea pig, rabbit or mouse C can be directly activated by oncornaviruses. Similar activation of C in the absence of antibody has been reported in other systems^{15,16}. Whether serum factors other than C or antibody are involved is not known. If oncornaviruses do infect man, lysis of these viruses may represent a natural resistance mechanism which would limit horizontal oncornavirus transmission and serve to maintain vertically transmitted oncornaviruses in a repressed, defective or latent state. It would be interesting to examine the status of the C system

in individuals with neoplasia and to determine the incidence of malignancies and oncornavirus expression in humans with deficiencies in the C system.

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Presence of antibody to a primate RNA virus in human plasma

AN antigen is present in leukocytes of patients with chronic myelogenous leukaemia (CML) which cross reacts with an antigen of the core protein of an RNA virus originally isolated from a Rhesus monkey, the Mason-Pfizer monkey virus (MPMV). This finding depended on development of a radioimmune assay using rabbit antibody raised against MPMV¹. I report here that using a modified Farr technique² plasma from some patients with various malignancies has antibody which reacts the MPMV core protein. The inference is that individuals in the human population may have been infected with a virus which is identical with or has antigens similar to those of the MPMV virus. The possible relevance of such infections to development of malignancies has yet to be determined.

A major protein of MPMV having a molecular weight of 25,000 (p25) was purified by a combination of Agarose gel filtration and DEAE-cellulose column chromatography. For a typical purification experiment, 10 ml of concentrated MPMV (5×10^{10} - 10^{11} particles ml⁻¹) grown in NC-37 cells were disrupted by exposure to 1% NP40 at 4 °C for 2-3 h. The mixture was separated on an Agarose A 0.5 M (BioRad) column (1.5 cm × 90 cm) equilibrated with 0.1% NP-40 in TNE (0.01 M Tris-HCl (pH 8.3), 0.15 M NaCl, 0.002 M ethylenediamine tetra-acetate) buffer. The peak containing p25 was applied to a DEAE-cellulose column (1.5 cm × 15 cm) equilibrated with 0.02 M phosphate buffer (pH 7.5) and eluted with 0.15 M sodium chloride. The isolated p25 was more than 95% pure as estimated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. The p25 was iodinated with ¹²⁵I by the chloramine T procedure³. A goat anti-rabbit antiserum gave a maximum of 72% indirect precipitation of a mixture of ¹²⁵I-p25 and a rabbit antiserum prepared against MPMV. This indirect radio-precipitation was inhibited by MPMV virus obtained from

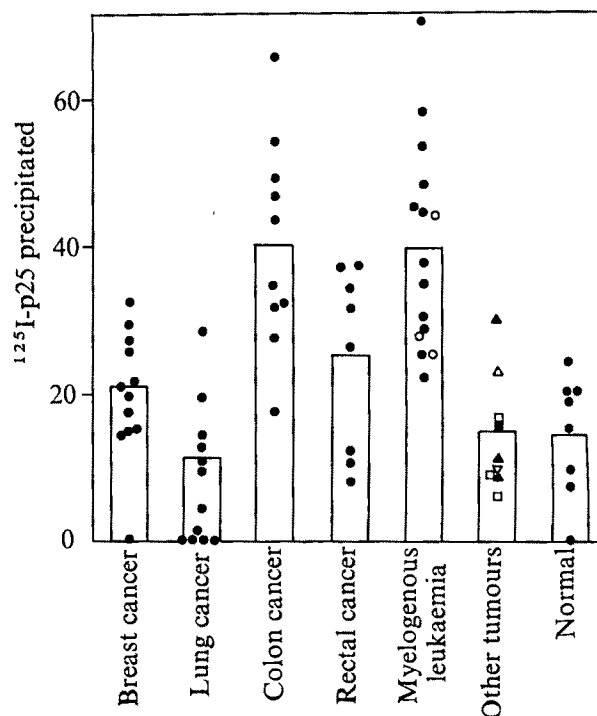


Fig. 1 The levels of antibody in each plasma are represented by the symbols. The individuals studied have been arbitrarily grouped in broad categories as indicated; the height of the bar represents the mean for each group. Myelogenous leukaemia group: ●, AML; ○, CML. Other tumours: ■, melanoma; □, osteosarcoma; ▲, carcinoma of larynx; ▽, acute lymphatic leukaemia; △, liposarcoma. Aliquots of heparinised plasma (200 µl) were incubated with ¹²⁵I-MPMV p25 and precipitated with 14% Na₂SO₄ as described in the legend of Table 1. The amount of ¹²⁵I-p25 precipitated by a plasma was converted to the percentage of ¹²⁵I-p25 precipitated assuming that the rabbit anti-MPMV precipitated 100% and the rabbit anti-MMTV precipitated 0%.

either cultures of human (NC-37) or simian (MT) cells infected with the virus. Proteins obtained from viral density region of disrupted non-infected NC-37 or simian cells (that is, in the region of 1.15-1.19 g ml⁻¹ in a sucrose density gradient) did not, however, cause any detectable inhibition of precipitation. Apparently then, the p25 is a viral protein and not a membrane component of the host cell, a finding in agreement with previous reports⁴.

In preliminary observations the conditions for an assay to be used for a survey of human plasmas were set. The percentage of ¹²⁵I-p25 precipitated with 14% sodium sul-

Table 1 ¹²⁵I-MPMV p25 bound to 14% sodium sulphate precipitates of human plasma and control antisera

Plasma	% bound to 14% Na ₂ SO ₄ precipitates of plasma			
	1:16	1:8	1:4	1:2
AML*	24.0	35.8	46.4	54.3
Normal individuals	12.4	13.4	15.5	16.8
Rabbit anti-MPMV	68.0	71.6	71.4	74.3
Rabbit anti-MMTV	NT	NT	12.8	13.2

*AML, Acute myelogenous leukaemia. NT, not tested.

The volume of each sample was adjusted to 400 µl with 0.1% BSA in TNE buffer. ¹²⁵I-MPMV p25, 2-3000 c.p.m. of specific activity of 5×10^6 c.p.m. µg⁻¹ was added to the sample and incubated 1 h at 37 °C. Final concentration of 14% Na₂SO₄ was made with 25% Na₂SO₄ solution followed by an additional 1 h incubation at 37 °C. Two-thirds of the supernatants (sup) were counted separately from the precipitates (ppt) with remaining supernatants. Then the percentage precipitation was calculated: % ppt = 100 [(ppt + 1/3 sup) - 1/2 (2/3 sup)] / [(ppt + 1/3 sup) + (2/3 sup)]. Aggregation of ¹²⁵I-p25 was minimised by storing in TNE buffer containing 10% glycerol and 1% Triton X-100. ¹²⁵I was prepared fresh every third week for the assays. In these conditions less than 4% of p25 was precipitable by 14% Na₂SO₄ without added plasma.

Table 2 Specific bindings of ^{125}I -MPMV p25 to purified human or rabbit IgG-coupled Sepharose columns

	Bindings of ^{125}I -P25 to IgG-Sepharose columns (c.p.m.)			Bindings of ^{125}I -BSA to IgG-Sepharose columns (c.p.m.)		
	RMTV	LHP	RMPV	RMTV	LHP	RMPV
Bound after wash	1,183	10,998	32,411	524	1,300	1,368
Elution by Glycine-HCl (pH 2.3)	210	2,895	13,342	182	309	296
Bound after Glycine-HCl (pH 2.3)	916	7,620	18,384	791	943	1,179
Elution by 6 M Guanidine-HCl	NT	2,087	10,921	NT	218	215
Bound after 6 M Guanidine-HCl	NT	6,500	8,775	NT	880	1,015
p25 Bound after wash	NT	NT	NT	NT	14,374	27,128

RMTV: rabbit anti-MMTV (mouse mammary tumour virus); LHP: plasma from a patient with acute myelogenous leukaemia; RMPV: rabbit anti-MPMV.
NT, not tested.

phate was measured using dilutions of plasma ranging from 1:2 to 1:16 (Table 1). Accurate quantitation of antibody present in large amounts in the rabbit anti-MPMV serum would require high dilutions of plasma. However, a plasma dilution of 1:2 would permit qualitative demonstration of antibody in plasmas containing either a great deal or only small amounts of antibody to ^{125}I -p25 as shown for two human sera (Table 1).

The binding of ^{125}I -p25 is at least partially due to IgG in the plasmas as shown in the following way. IgG was purified from various rabbit and human plasmas by successive 18% and 14% Na_2SO_4 precipitations followed by DEAE-cellulose column chromatography. A single typical IgG precipitin arc was obtained for each preparation on immunoelectrophoresis using appropriate goat anti-human or anti-rabbit serum. Forty milligrams of each purified Ig was coupled to 4 ml of CNBr activated Sepharose. For each immunoabsorbent column, 1 ml of Sepharose was used. Radiolabelled antigens (^{125}I -p25, 51,000 c.p.m.; ^{125}I -BSA, 57,000 c.p.m.) were added to the column and incubated for 2 h at room temperature. The Sepharose columns were washed with $\times 20$ volumes of 0.2% ovalbumin in TNE buffer to remove unbound radiolabelled antigens. Successive elutions were made with 5 ml of 0.1 M glycine-HCl buffer (pH 2.3) and 6 M guanidine-HCl. As shown in Table 2, the purified Ig which was obtained from whole plasma bound ^{125}I -p25 specifically.

The survey of plasmas for antibody against ^{125}I -p25 was done on samples from 75 individuals, 67 of whom had a clinical diagnosis of malignancy and eight of whom were apparently healthy volunteers including three normal pregnant women. The samples were obtained from patients at Billings Hospital, University of Chicago and Presbyterian Hospital, Columbia University, New York. These donors were selected without regard to age, sex, stage or type of the malignancy or therapy.

The percentage of ^{125}I -p25 bound by each plasma is plotted in Fig. 1. The individual responses for 8 normal volunteers, 8 patients with carcinoma of the rectum, 12 patients with carcinoma of the lung, 13 patients with carcinoma of the breast and 9 patients with other malignancies range from 15 to 25% binding of ^{125}I -p25; differences between the means of these groups are not statistically significant ($P > 0.05$). Patients with carcinoma of the colon and leukaemia, however, had levels as high as 66–70% binding, furthermore, plasmas from only single individuals in each of these groups have less antibody than the normal individual with the highest level of binding. The significance levels of differences between the means are: $P < 0.005$ for patients with carcinoma of the colon and the normal group and $P < 0.001$ for patients with leukaemia and the normal group.

These results suggest that some individuals may have

been or are infected with a virus similar to or identical with MPMV. The demonstration of an antigen in leukocytes of patients with CML¹ is consistent with this possibility. In so far as it has been examined, antigens cross reactive with p25 of MPMV have not been found in other C type RNA viruses, but such cross reactions might be found on more extensive survey using highly sensitive radioimmune assays.

Patients with leukaemia or carcinoma of the colon may simply be more prone to exposure and/or infection with the agent responsible for inducing antibody against p25 of MPMV. Alternatively, of course, the infection may be relevant to development of malignancy. MPMV-like particles have been identified in human cell lines derived from a leukaemia or other malignancies^{5–7}, as well as from spontaneously transformed normal cells^{8,9}. The MPMV has morphological, biochemical and biophysical characteristics of known oncornaviruses^{10,11} though they have not been shown to share immunologic characteristics^{4,8}. Considerable evidence now supports the general proposition that viruses have aetiological significance in leukaemogenesis in humans¹² as well as in mice¹³. In mice, humoral antibodies to endogenous or exogenous leukaemia viruses presumably reflect exposure to the virus^{14,15}; the demonstration here of antibody to p25 of MPMV may have analogous significance. This possibility might be best studied in humans with CML, since leukocytes from such patients contain a protein antigenically cross reactive with p25 of MPMV and plasma from many such patients contains considerable antibody to the antigen. Furthermore, long remissions of the disease often occur. Therefore, in studies under way, changes which may occur in levels of the leukocyte antigen and in plasma levels of antibody will be studied during the course of the disease.

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Discrimination between eukaryotic and prokaryotic, and formylated and non-formylated, initiator tRNAs by eukaryotic initiation factor EIF-3

PROKARYOTIC and eukaryotic cells contain two methionine accepting species of transfer RNA, tRNA^{Met} and tRNA^{Met} prokaryotes⁴ and non-formylated Met-tRNA_i in prokaryotes⁴ and non-formylated Met-tRNA_i from eukaryotes⁵⁻⁹ act exclusively in chain initiation; Met-tRNA_m provides amino acids for internal positions in proteins⁴⁻⁹. Thus the structure of tRNA^{Met} is likely to have some unique attribute that makes it specific for initiation and that provides for its recognition by initiation factors¹⁰⁻¹³. We have investigated the specificity of an eukaryotic initiation factor (EIF-3) for initiator tRNA. A preparation of EIF-3 formed a complex preferentially with nonformylated eukaryotic Met-tRNA_i; the factor discriminated against the formylated eukaryotic species and against prokaryotic initiator tRNA whether it was formylated or not. A preliminary account of the experiments has been reported before¹⁴.

We have isolated EIF-3 from mouse Krebs II ascites cells by chromatography of a 0.25 M KCl ribosomal salt wash on DEAE-Sephadex and hydroxylapatite (details of the isolation of EIF-3 will be presented elsewhere). EIF-3, like initiation factor IF-2 from *E. coli*¹¹⁻¹², forms a ternary complex with GTP and eukaryotic Met-tRNA_i; the ternary complex is quantitatively retained on Millipore filters. The factor does not form a ternary complex with Met-tRNA_m or other tRNA species. A similar factor has been prepared from rabbit reticulocytes and L cells¹⁵⁻¹⁸. The preformed ternary complex is transferred to 40S ribosomal subunits in the absence of mRNA. The nature of the partial reactions catalysed by EIF-3 (that is the formation of the ternary complex and its transfer to 40S ribosomal subunits) suggests that the factor is involved in the recognition of Met-tRNA_i, and is therefore an intermediate in the formation of a 40S preinitiation complex.

We compared the recognition by EIF-3 of eukaryotic and prokaryotic Met-tRNA_i and fMet-tRNA_i. EIF-3 formed a ternary complex with GTP and yeast or ascites cell Met-tRNA_i very rapidly (Fig. 1); however, there was little (only about 15% as much) ternary complex formation when *E. coli* Met-tRNA_i was used (Fig. 1). Formylated prokaryotic Met-tRNA_i was an even poorer substrate for EIF-3 (Fig. 1). Similar results were obtained when complex formation was measured as a function of the amount of EIF-3 (Fig. 2).

We next investigated whether EIF-3 distinguished between formylated and non-formylated Met-tRNA_i (Table 1). Although EIF-3 clearly used non-formylated eukaryotic (yeast or ascites cell) Met-tRNA_i preparations more efficiently than the formylated variety, it appeared that the factor would form a complex with the formylated species, albeit only 35-50% as well (Table 1). The formylation reaction was, however, only 65-80% effective; thus complex formation could have been with contaminating non-formylated Met-tRNA_i. Indeed, when a preparation of ascites cell fMet-tRNA_i that contained 9,400 c.p.m. and was 64% formylated was incubated with GTP and increasing amounts (up to 20 µg) of EIF-3, the radioactivity in the ternary complex, when a plateau was reached, was 4,300 c.p.m.; binding was maximum with less than 10 µg of EIF-3

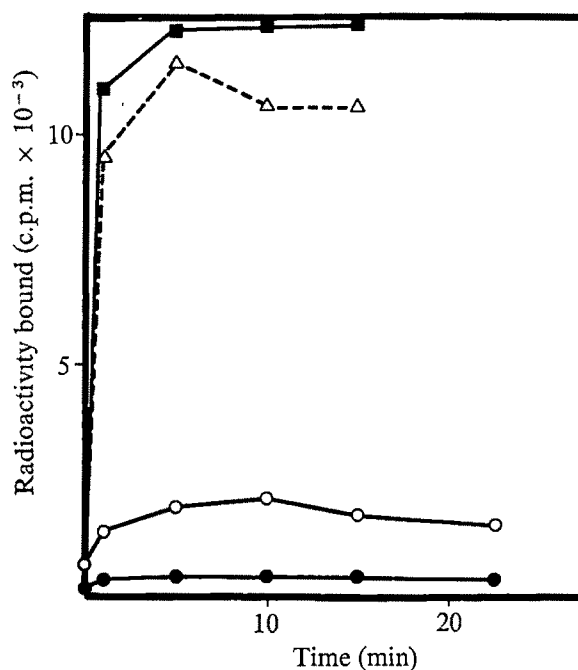


Fig. 1 Kinetics of ternary complex formation with EIF-3, GTP and eukaryotic or prokaryotic initiator tRNA. The reaction mixture for the eukaryotic initiator tRNA experiments contained in 100 µl of buffer A (20 mM Tris-HCl, pH 7.6; 100 mM KCl; 0.2 mM magnesium acetate, and 1 mM dithiothreitol): 34.2 µg of EIF-3; 0.2 mM GTP; and 200 µg of ascites cell tRNA containing 76,000 c.p.m. of ³H-Met-tRNA_i, or 125 µg of yeast tRNA containing 65,000 c.p.m. of ³H-Met-tRNA_i. For the prokaryotic initiator tRNA experiments, 150 µl of buffer A contained: 51.3 µg of EIF-3; 0.2 mM GTP, and 19 µg of *E. coli* tRNA^{Met} with 66,600 c.p.m. of ³H-Met-tRNA_i or 17.3 µg of *E. coli* tRNA^{Met} with 74,000 c.p.m. of formyl-³H-Met-tRNA_i. Samples were incubated at 30 °C; at intervals, 25-µl samples were removed and the formation of a ternary complex determined by filtration on Millipore filters. The filters were washed three times with 3 ml of buffer A. The filters were dried and placed in glass vials containing 5 ml of scintillation fluid¹⁹. Radioactivity was determined in a Packard Tri-Carb spectrometer with an efficiency of 16%. The tRNA^{Met} species was prepared from unfractionated ascites cell tRNA or from yeast tRNA by chromatography on BD-cellulose⁶. The purified *E. coli* tRNA^{Met} was obtained from Oak Ridge National Laboratory. The tRNA was charged with ³H-methionine (4.3 Ci mmol⁻¹) using a preparation of *E. coli* aminoacyl-tRNA synthetases^{3,20}. When ³H-Met-tRNA_i was to be formylated, tetrahydrofolic acid (Leucovorin, Lederle Laboratories) was included in the reaction mixture²¹ and the extent of formylation was determined by paper chromatography of a deacylated sample²¹. ■, Yeast Met-tRNA_i; △, ascites Met-tRNA_i; ○, *E. coli* Met-tRNA_i; ●, *E. coli* fMet-tRNA_i.

(results not shown). The results are by no means conclusive, but since EIF-3 prefers the non-formylated species they are consistent with complex formation having occurred predominantly, if not exclusively, with the non-formylated Met-tRNA_i that was present.

We sought to confirm our impression that it was chiefly non-formylated Met-tRNA_i contaminating preparations of fMet-tRNA_i that was responsible for ternary complex formation (Table 2). The complex formed with EIF-3, GTP and partially (64%) formylated Met-tRNA_i was isolated on Millipore filters and digested with pancreatic RNase. The amount of Met-tRNA_i, as methionyl-adenosine and fMet-tRNA_i, as formyl-methionyl-adenosine²², in the RNase digest was determined after extraction with ethyl acetate²³. Only about 14% of the ternary complex contained fMet-tRNA_i. The predominant species in the complex (85-90%) was Met-tRNA_i. Cashion and Stanley²⁴ have reported similar results: they found a like amount (10%) of ternary complex formation when formylated eukaryotic Met-tRNA_i, rather than the non-formylated species, was tested with an initiation factor (IF_i) from rabbit reticulocytes.

Table 1 Discrimination by EIF-3 between eukaryotic and prokaryotic initiator tRNA

Initiator tRNA	Aminoacyl-tRNA bound (pmol)
Met-tRNA _f (ascites)	3.53
fMet-tRNA _f (ascites) (70% formylated)	1.28
Met-tRNA _f (yeast)	3.55
fMet-tRNA _f (yeast) (80% formylated)	1.80
Met-tRNA _f (<i>E. coli</i>)	0.59
fMet-tRNA _f (<i>E. coli</i>) (99% formylated)	0.11

The reaction mixture (25 μ l in buffer A) contained: 5.7 μ g of EIF-3; 0.2 mM GTP; and 40 μ g of ascites cell tRNA containing 13,800 c.p.m. of ³H-Met-tRNA_f, or 84 μ g of ascites cell tRNA containing 10,400 c.p.m. of formyl-³H-Met-tRNA_f, or 25 μ g of yeast tRNA containing 13,000 c.p.m. of ³H-Met-tRNA_f, or 24 μ g of yeast tRNA containing 16,000 c.p.m. of formyl-³H-Met-tRNA_f, or 3.1 μ g of *E. coli* tRNA_f^{Met} containing 11,100 c.p.m. of ³H-Met-tRNA_f, or 2.9 μ g of *E. coli* tRNA_f^{Met} containing 12,400 c.p.m. of formyl-³H-Met-tRNA_f. Incubation was at 30 °C for 5 min and the complex was assayed on Millipore filters.

Once again there was little ternary complex formation with EIF-3, GTP and prokaryotic Met-tRNA_f; however, even that small amount was reduced if *E. coli* Met-tRNA_f was formylated (Table 1).

The eukaryotic initiation factor EIF-3 shows a clear preference for non-formylated eukaryotic Met-tRNA_f. Formylation decreases the ability of the eukaryotic initiator tRNA to serve as a substrate for EIF-3, and prokaryotic Met-tRNA_f, whether formylated or not, serves especially poorly in the reaction. The observations are consistent with the finding reported by Heckman *et al.* (J. Heckman, G. Temple, C.L. Woodley, N.K. Gupta and U.L. RajBhandary, personal communication) that prokaryotic Met-tRNA_f is much less efficient (20–30%) than eukaryotic Met-tRNA_f in the translation of haemoglobin mRNA in mouse Krebs II ascites extracts. It is likely that the results reflect differences in the structure of eukaryotic and prokaryotic initiator tRNAs, initiation factors, or both. There is no extensive homology in the nucleotide sequence of initiator tRNAs from *E. coli*²⁵ and eukaryotes^{26–28}, and the initiation factors are almost certainly different.

The efficient formation of a ternary complex between mouse ascites cell EIF-3 and Met-tRNA_f from yeast, a phylogenetically distant eukaryote, implies conservation of the recognition sites. Indeed, the results were to be expected since the nucleotide sequence of initiator tRNAs from higher eukaryotes (rabbit liver²⁷, sheep mammary gland²⁷ and mouse P3 myeloma²⁸) have extensive homology with yeast initiator tRNA²⁶.

Lodish *et al.*²⁹ had reported that yeast fMet-tRNA_f was

used as effectively as Met-tRNA_f in initiation of protein synthesis by rabbit reticulocyte ribosomes. On the other hand, other available results^{8,30,31} more nearly approximated ours: that the incorporation of formylmethionine from eukaryotic fMet-tRNA_f into polypeptides in mouse Krebs II ascites extracts was slow and never very extensive when compared with that with Met-tRNA_f. The conflict may reflect to some degree difference in the cells used, but it may be important that in the experiments of Lodish *et al.*²⁹ high concentrations of purified initiator tRNA were used, concentrations so high as to risk dampening the differences in efficiency of incorporation from Met-tRNA_f and fMet-tRNA_f. The observations emphasise the great importance of using physiologically proper substrates for studying the partial reactions in the initiation of protein synthesis.

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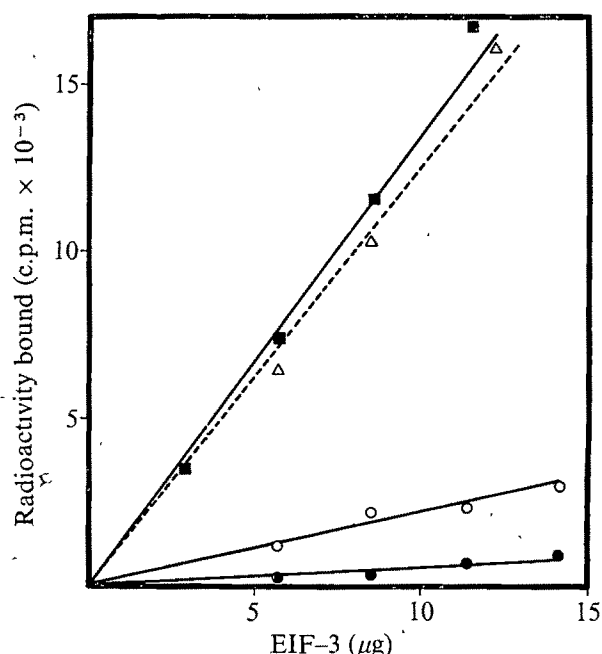


Fig. 2 Effect of the amount of EIF-3 on the formation of a ternary complex with eukaryotic or prokaryotic initiator tRNA. The reaction mixture (25 μ l in buffer A) contained: EIF-3 (as indicated); 0.2 mM GTP; and 100 μ g of ascites cell tRNA containing 25,000 c.p.m. of ³H-Met-tRNA_f, or 37.5 μ g of yeast tRNA containing 19,500 c.p.m. of ³H-Met-tRNA_f, or 2.9 μ g of *E. coli* tRNA_f^{Met} containing 12,400 c.p.m. of formyl-³H-Met-tRNA_f, or 3.1 μ g of *E. coli* tRNA_f^{Met} containing 11,000 c.p.m. of ³H-Met-tRNA_f. Incubations were at 30 °C for 5 min. Samples were assayed as described in Fig. 1. Symbols as in Fig. 1.

Table 2 Ternary complex formation with EIF-3 and fMet-tRNA_f or Met-tRNA_f

Sample	Met-tRNA _f or fMet-tRNA _f bound to Millipore filter (pmol)	Met-tRNA _f or fMet-tRNA _f remaining bound to Millipore filter after RNase digestion (pmol)	fMet-tRNA as fMet-adenosine in ethyl acetate phase (pmol)	Met-tRNA _f as Met-adenosine in aqueous phase (pmol)
Control	1.94	—	—	—
Digested with RNase and extracted with ethyl acetate	—	0.30	0.19 (13.7%)	1.20 (86.3%)

The reaction mixture (25 μ l in buffer A) contained: 7.6 μ g of EIF-3; 59.5 μ g of tRNA containing 12,200 c.p.m. of ³H-fMet-tRNA_f (64% formylated); and 0.2 mM GTP. Incubation was for 5 min at 30 °C. The EIF-3·GTP·Met-tRNA_f or EIF-3·GTP·fMet-tRNA_f complex was collected on Millipore filters. One group was used to determine the total amount of complex formed; the other was digested with (6 μ g) pancreatic RNase in 1 ml of 0.1 M potassium acetate buffer, pH 5.5 at 30 °C for 15 min. The complex remaining bound to Millipore filters was assayed (see Table). The fMet-tRNA_f as fMet-adenosine²² in potassium acetate buffer (pH 5.5) was extracted with ethyl acetate according to Leder and Bursztyn²³ and the Met-tRNA_f as Met-adenosine²³ remaining in the aqueous phase was determined. The values are the average of duplicate samples.

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Blocked, methylated 5'-terminal sequence in avian sarcoma virus RNA

mRNAs of many eukaryotic cells and viruses contain blocked, methylated 5'-terminal structures of the type, m⁷GpppN (ref. 1). This 'cap' structure, and in particular the 5'-terminal 7-methyl-guanosine, is required for efficient translation *in vitro* of globin, reovirus and vesicular stomatitis virus mRNAs^{2,3}. The genome subunits of RNA tumour viruses probably function as viral mRNA since RNA of the same base sequence is present in virus-specific polysomes of infected cells^{4,5} and virion genome RNA can be translated *in vitro* in a eukaryotic system⁶. Consistent with this possibility, we have found a blocked, methylated 5'-terminal structure—m⁷GpppG^mpCp—in the genome RNA of avian sarcoma virus (ASV).

Subunits of ³²P-labelled B77-ASV genome RNA were isolated by rate-zonal centrifugation of denatured viral RNA as described previously⁷. RNA from about the 35S region of the gradient migrated in the vicinity of 35S poliovirus RNA when analysed by polyacrylamide gel electrophoresis. The latter procedure resolved the a (35S) and b (smaller than 35S) RNA subunits which correspond to transforming and transformation defective particles⁸ present in the virus stock at levels of 20% and 80% respectively. The RNA hybridised completely with virus-specific DNA synthesised *in vitro* (data not shown).

To test for the presence of blocked 5' termini in ASV genome RNA, we used a procedure developed originally for the isolation of 3'-terminal oligonucleotides from high molecular weight RNA⁹. It depends on the selective affinity of 2'-3' *cis* diols for

DBAE-cellulose, a derivative with covalently bound dihydroxyboryl groups. ASV ³²P-labelled genome RNA was mixed with ³H-methyl-labelled reovirus mRNA, digested to completion with RNase T₂, and applied to a column of DBAE-cellulose. The 5'-terminal RNase T₂ fragment of reovirus mRNA, m⁷G(5')ppp(5')G^mpCp, contains all the methyl groups of the viral RNA and free 2',3'-hydroxyl groups in the 5'-terminal m⁷G (ref. 10). Consequently, the ³H-methyl radioactivity bound quantitatively to DBAE-cellulose in buffer A (Fig. 1). Most of the ³²P-labelled digestion products consisted of 3' mononucleotides and did not bind; however, 0.08% of the ³²P bound to the resin and, after changing the buffer, eluted with the ³H-labelled m⁷GpppG^mpCp. The 3'-terminal adenosine released by RNase digestion¹¹ presumably bound but contained no ³²P and was undetected. This value is in excellent agreement with the expected amount of DBAE-bound ³²P calculated for approximately 35S RNA (7,500 nucleotides) containing a RNase T₂-resistant, 5'-5'-linked cap structure similar to that in reovirus RNA¹⁰ (5 phosphates).

The RNase T₂ digested mixture of ³²P-labelled ASV fragments and ³H-labelled m⁷GpppG^mpCp was eluted from DBAE-

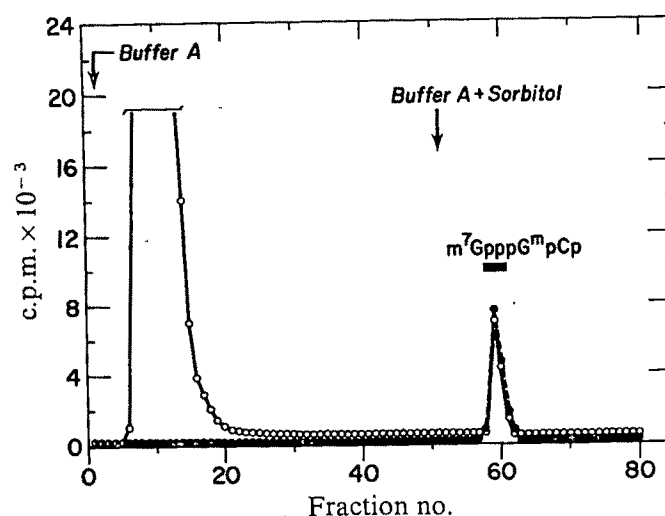


Fig. 1 Chromatography of RNase T₂ digest of ³²P-labelled ASV genome RNA on DBAE-cellulose. The B77 strain (sub-group C) of ASV was propagated in chick embryo fibroblasts, isolated by centrifugation and the viral RNA extracted with Pronase-sodium dodecyl sulphate-phenol, all as described previously⁷. Growth medium (phosphate-free) containing dialysed calf serum (see ref. 7 for details) and ³²P-orthophosphate (2 mCi ml⁻¹) was changed every 12 h for a total of three collections; viral RNA labelled in this manner had a specific activity of 15 × 10⁶-30 × 10⁶ c.p.m. μg⁻¹. The 70S genome RNA was isolated by rate-zonal centrifugation⁷, dissolved in 0.01 M EDTA-0.02 M Tris-HCl, pH 7.4-0.5% (w/v) SDS, heated at 80 °C for 4 min and centrifuged through a gradient of 15-30% sucrose containing 0.1 M NaCl-0.01 M EDTA-0.02 M Tris-HCl, pH 7.4-0.5% SDS. Centrifugation was in a Spinco SW 27.1 rotor at 25,000 r.p.m. for 15 h at 20 °C. The gradient was fractionated and radioactivity in the fractions measured as Cerenkov radiation. ASV genome RNA, 1.1 × 10⁷ c.p.m., was mixed with ³H-methyl-labelled reovirus mRNA synthesised *in vitro* (10 μg, 1 × 10⁵ c.p.m.) and digested with 50 units RNase T₂ (Sankyo) in 250 μl of 10 mM sodium acetate buffer (pH 4.5) containing 1 mM EDTA. After incubation for 6 h at 37 °C, the reaction mixture was adjusted to starting buffer A (0.05 M morpholine, pH 8.7, 1.0 M NaCl, 0.1 M MgCl₂, 20% dimethylsulphoxide) in a total volume of 1 ml. The mixture was applied on to a DBAE-cellulose column (0.6 × 15 cm) equilibrated with buffer A (ref. 9). Elution was carried out with buffer A at a flow rate of 5 ml h⁻¹. When most of the radioactive material had been eluted from the column, the buffer was abruptly changed to buffer A plus 1 M sorbitol and elution continued at a flow rate of 6-7 ml h⁻¹. ³²P radioactivity was determined by Cerenkov counting and ³H radioactivity measured by dissolving an aliquot (25 μl) of each fraction in 1 ml methyl-cellosolve and 10 ml Aquasol (New England Nuclear). ○, ³²P; ●, ³H.

Table 1 Methylated nucleosides derived from 70S and 4S ASV RNA

	70S-I	Free 4S	70S-II	35S
m ⁷ G	13*	18	20	2
mC	14	17	21	3
mA	—	1	—	—
mG+mU	36	64	59	10
cap m ⁷ G	5	0	—	14
cap G ^m	5	0	—	
m ⁶ A	27	—	—	71
Total c.p.m.	4,227	10,689	2,654	1,048

*Value given in % of total ³H-methyl radioactivity.

Two 100-mm plates of transformed, B77-infected chick cells were incubated for 10 h in 5 ml of amino acid-free medium containing dialysed serum, 10⁻⁴ M each of adenosine and guanosine, and 500 μ Ci ml⁻¹ of ³H-methyl-methionine (specific activity 10.5 Ci mmol⁻¹, New England Nuclear). The medium was collected, fresh medium of the same composition but lacking radioisotope was added, and collected after 10 h and pooled with the first collection. The virus was collected by pelleting, disrupted in 0.02 M Tris buffer, pH 7.4, containing 0.15 M NaCl, 0.001 M EDTA and 0.5% sodium dodecyl sulphate and the 70S and 4S RNA separated by density gradient centrifugation⁷. The 35S RNA was obtained from 70S RNA after denaturation and centrifugation as described for Fig. 1. The RNA-containing fractions were pooled, precipitated with ethanol, digested with *Penicillium* nuclease and alkaline phosphatase, and analysed by paper electrophoresis at pH 3.5 as described in Fig. 2 (ref. 10). The radioactivity in the region corresponding to methylated derivatives of adenosine in the 70S RNA digest was eluted and identified by paper chromatography with authentic marker N⁶-methyladenosine in two different solvents: isobutyric acid–0.5 M NH₄OH (10:6 v/v) and isopropanol–concentrated NH₄OH–H₂O (7:1:2 v/v). The nuclease- and phosphatase-resistant radioactivity in presumptive caps of 70S RNA was eluted, treated with nucleotide pyrophosphatase and alkaline phosphatase and the digest was analysed by paper electrophoresis and identified as consisting of equal amounts of m⁷G and G^m by subsequent paper chromatography with authentic marker compounds¹⁸. For comparison of the composition of free and 70S genome-associated 4S RNA, values in column (70S-II) were calculated from (70S-I) after omitting the radioactivity in caps and N⁶-methyladenosine. mC, mA, mG, mU are methylated derivatives of the corresponding nucleosides that were not identified further.

cellulose, desalted and reanalysed by DEAE–cellulose chromatography to determine its net charge¹⁰. Two peaks of ³²P with net charges of –4.5 and –5 were resolved, and both contained a corresponding proportion of ³H-methyl radioactivity (Fig. 2). The fractions comprising the major peak were pooled, desalted, digested with *Penicillium* nuclease (P₁) and analysed by paper electrophoresis. P₁ hydrolyses phosphodiester linkages, including those containing 2'-O-methylated residues, to yield 5' mononucleotides from polynucleotides. The enzyme also has 3' phosphatase activity, but cannot cleave cap structures^{10,12}; thus, ³H-methyl-labelled m⁷GpppG^mpCp, derived from reovirus mRNA by RNase T₂ digestion, yielded all the radioactivity as m⁷GpppG^m after P₁ treatment (Fig. 2). By contrast, the ³²P-labelled material yielded radioactivity in the positions of pC, m⁷GpppG^m and Pi in a ratio close to 1:3:1 as expected for P₁ cleavage of m⁷GpppG^mpCp.

Another preparation of RNase T₂ 5'-terminal fragments was digested with P₁ and alkaline phosphatase and analysed by electrophoresis (Fig. 3a). The ³H-methyl-labelled 5'-terminal m⁷GpppG^mpCp was converted to m⁷GpppG^m. The ³²P-labelled fragments yielded 60% of the radioactivity in the position of m⁷GpppG^m and 40% as Pi. The ³H-labelled m⁷GpppG^m and the comigrating ³²P-labelled material were eluted and analysed by descending paper chromatography (Fig. 3b). All the ³H and ³²P radioactivity again comigrated, and the ratio of ³²P to ³H was constant across the peak. The position of the major peak of ³²P corresponded to that of m⁷GpppG^m. The minor peak (fractions 12–14) seemed to result from partial degradation during DBAE–cellulose chromatography at pH 8.7. This material and the smaller peak in Fig. 2a (fractions 90–95) consisted of the ring-opened derivative of m⁷GpppG^m, as determined by electrophoresis and chromatography of nucleotide pyrophosphatase digests. The major peak of radioactivity

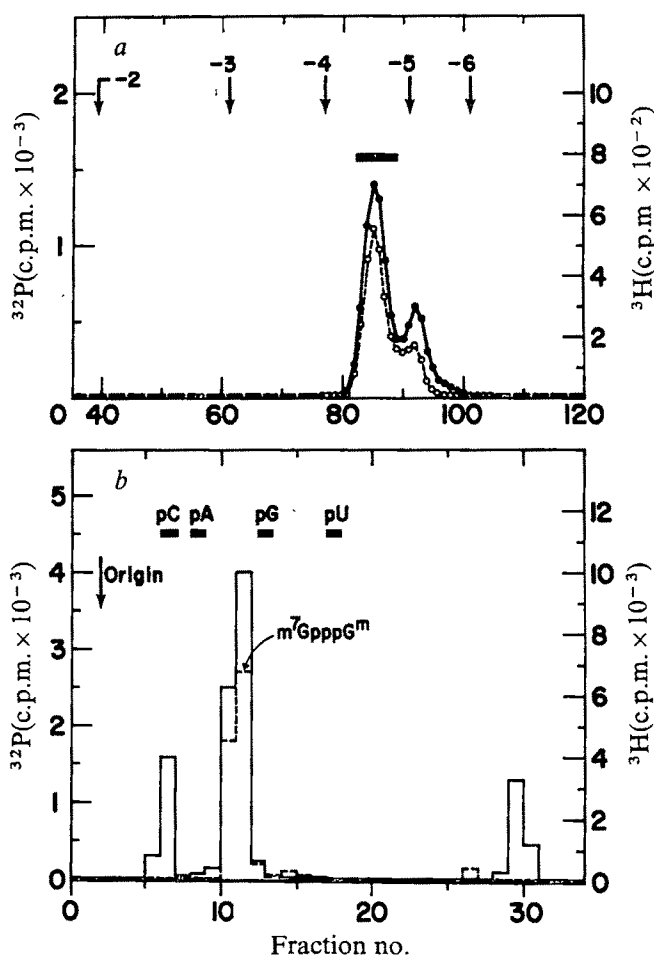


Fig. 2 Characterisation of blocked 5'-terminal fragment from ASV genome RNA. *a*, The peak fractions eluted with buffer A containing 1 M sorbitol (Fig. 1) were diluted tenfold with water and applied to a DEAE–cellulose column (0.6×40 cm) equilibrated with 0.02 M Tris–HCl (pH 8.0) and eluted with a linear gradient of NaCl (0.05–0.3 M) in 0.02 M Tris–HCl buffer (pH 8.0) containing 7 M urea (total 150 ml). A pancreatic RNase digest of yeast tRNA was included as marker for the oligonucleotide elution positions. ³²P (●), and ³H (○) radioactivity were determined as described in Fig. 1. *b*, Peak fractions 82–88 were pooled as indicated, desalted, dissolved in 200 μ l of 5 mM sodium acetate buffer (pH 6.0) and digested with 100 μ g of P₁ nuclease at 37 °C for 30 min. The digest was analysed by high voltage paper electrophoresis in pyridine acetate buffer (pH 3.5) at 50 V cm⁻¹ for 50 min (ref. 10). ³²P, (—); ³H, (---)

(fractions 16–19) was eluted and an aliquot analysed by DEAE–cellulose chromatography; the ³H and ³²P eluted in one peak with a net charge between –2 and –3 (Fig. 3b, inset). The results are consistent with the presence of three phosphates in a phosphatase-resistant structure, previously demonstrated to be m⁷GpppG^m for reovirus mRNA 5' ends¹⁰. Similarly, when ³²P-labelled ASV genome RNA was digested with P₁ and alkaline phosphatase and analysed directly by electrophoresis, 0.04% of the total ³²P was obtained as a peak in the position of m⁷GpppG^m. This value is close to that calculated for one cap structure containing three protected phosphates per RNA molecule of chain length 7,500 nucleotides.

To determine if an identical 5' structure is present in ASV and reovirus RNA, an aliquot of P₁ and alkaline phosphatase-treated mRNA fragments (corresponding to fractions 16–19 in Fig. 3b) was digested with nucleotide pyrophosphatase and analysed by electrophoresis. The ³H-methyl radioactivity in m⁷GpppG^m was converted to equal amounts of m⁷pG and pG^m. The ³²P was resolved into three equally labelled constituents which migrated in the positions of m⁷pG, pG^m and Pi;

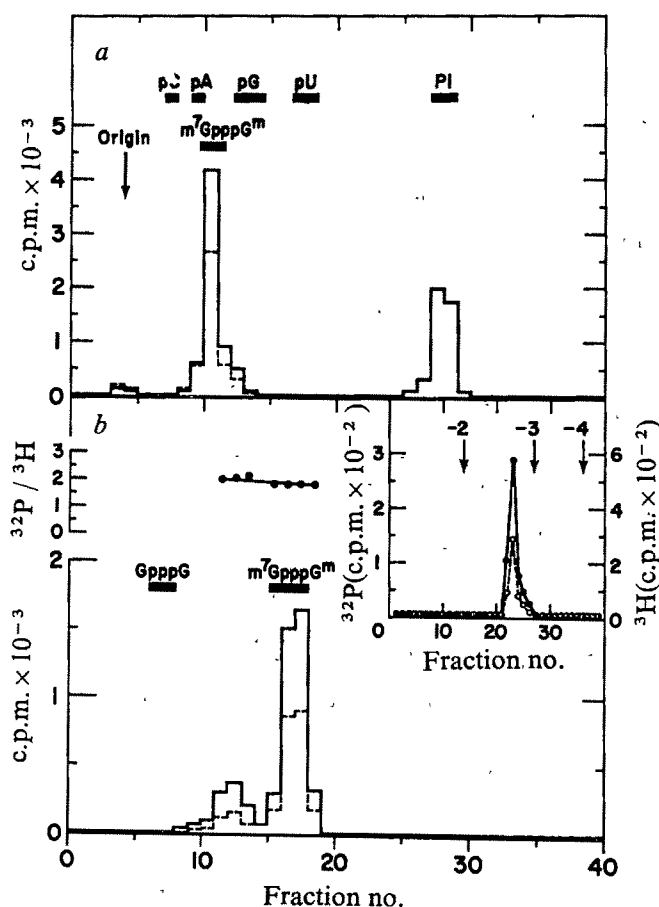


Fig. 3 Analysis of blocked 5'-terminal structure by electrophoresis and chromatography. Another preparation of 5'-terminal RNase T₂ fragments of ³H-methyl-labelled reovirus mRNA and ³²P-labelled ASV genome RNA was obtained as in Fig. 1. a, After desalting, the radioactive DBAE-cellulose-bound material was digested successively with P_i nuclease and alkaline phosphatase, and the digest analysed by paper electrophoresis; b, the enzyme-resistant radioactivity which migrated between pA and pG was extracted and chromatographed on paper using isobutyric acid-0.5 M NH₄OH (10:6 v/v) as a solvent. Inset, An aliquot of the material in fractions 16-19 of B was applied to a DEAE-cellulose column (0.6 × 25 cm) and eluted as described in Fig. 2a. ○, —, ³²P; ●, ---, ³H.

the nucleotides were eluted and further identified as m⁷pG and pG^m by paper chromatography. Thus the 5' termini of the ASV genome subunits probably have only one type of blocked, methylated structure, namely, m⁷GpppG^mpCp.

In addition to blocked, methylated 5'-terminal structures, poly(A)-containing RNA isolated from eukaryotic cells contains m⁶A in internal positions¹³⁻¹⁸. The same methylated nucleoside was found in ASV genome RNA (Table 1). Virion 70S RNA labelled with ³H-methyl-methionine contained 10% of the total radioactivity in m⁷GpppG^m and 27% in m⁶A, a methylated derivative of adenosine rarely found in tRNA¹⁹. Transfer (4S) RNA present in virions but not associated with the genome RNA contained no cap structures and only a low level of methylated adenosine; m⁷G and methylated derivatives of cytidine, guanosine and uridine were present as reported previously for 4S RNA in Rous sarcoma virus²⁰. The proportions of methylated nucleosides in free 4S RNA and 70S RNA were similar when calculated after subtracting the radioactivity in caps and m⁶A, consistent with the presence of the latter in genome subunit RNA (Table 1). Direct analysis of ³H-methyl-labelled approximately 35S RNA from denatured 70S RNA revealed that ASV genome RNA contained 71% of the total radioactivity in m⁶A and 14% in m⁷GpppG^m, sug-

gesting that there are about 10 residues of m⁶A per RNA subunit. On the basis that 4S RNA contains an average of 11 methylated nucleotides¹⁹, the number of 4S RNA molecules per genome subunit was estimated to be close to two.

ASV genome RNA, like cellular cytoplasmic mRNA, contains 5'-m⁷GpppN, 3'-terminal poly(A) and internal m⁶A and is probably formed by cellular mechanisms of mRNA biosynthesis. Poly(A)-containing RNA from the nuclei of HeLa cells also has m⁷GpppN at the 5' end¹⁸. It is not, however, known whether ASV genome RNA is 'capped' and methylated in the nucleus or in the cytoplasm of infected cells. Viral RNA may be made from larger precursor RNA by a processing mechanism involving concomitant addition of caps²¹. Alternatively, 'capping' of ASV genome RNA may occur at the initiation step of transcription in a manner analogous to mRNA synthesis and modification by virion-associated enzymes²²⁻²⁴. The same 5'-terminal sequence, m⁷GpppG^mpCp, has also been found in Prague strain Rous sarcoma virus 35S RNA (J. M. Keith and H. Fraenkel-Conrat, personal communication).

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Thermodynamic activation parameters of fish myofibrillar ATPase enzyme and evolutionary adaptations to temperature

INTERSPECIFIC compensatory adaptations to environmental temperature which occur at the molecular level have been demonstrated for several enzyme systems¹. Most of these studies have been concerned with either kinetic parameters such as *K_m* (refs 2, 3) or thermodynamic parameters such as activation energy^{2,4}. The significance of changes in these parameters in the overall mechanism of evolutionary temperature

Table 1 Thermodynamic activation parameters for fish myofibrillar ATPase activity

Species	Approximate environmental temperature (°C)	Assay temperature (°C)	V _{max} (μmol Pi mg ⁻¹ min ⁻¹)	E _a (calorie mol ⁻¹)	No. assays	ΔH [‡] (calorie mol ⁻¹)	ΔS [‡] (entropy units)	ΔG [‡] (calorie mol ⁻¹)
<i>Nothenia rossii</i>	South Georgia, British Antarctica (0°–2 °C)	0.5	0.24			6,850	–42.2	18,450
		18	0.81	7,400**	21	6,800	–39.4	18,300
<i>Gadus virens</i>	North Sea (5°–14 °C)	0.5	0.06			10,200	–33.7	19,400
		18	0.82	10,700*	18	10,100	–28.1	18,900
<i>Gadus morhua</i>	North Sea (5°–14 °C)	0.5	0.07			11,350	–26.2	18,500
		18	0.61	11,900***	24	11,300	–24.6	19,050
<i>Amphiprion sebae</i>	Indian Ocean (about 23°–25 °C)	0.5	0.013			16,750	–9.6	19,400
		18	0.57	17,300*	18	16,700	–6.1	18,500
<i>Carassius carassius</i>	Domestic (Acclimatised to 26 °C)	0.5	0.03			21,400	8.3	19,050
		18	0.35	21,900*	18	21,300	8.6	18,800
<i>Tipalia nigra</i>	Equatorial African freshwater lake (23°–31 °C)	0.5	0.017			31,000	42.9	19,300
		18	0.045	31,600**	18	31,500	42.5	18,600
<i>Tipalia grahami</i>	Equatorial hot springs soda lake (35°–38 °C)	0.5	0.016			32,800	49.2	19,300
		18	0.69	33,300***	18	33,300	49.2	19,000

Myofibrils were prepared from the dorsal epaxial musculature as before¹¹, care was taken to exclude superficial red muscle as this has a different myofibrillar ATPase activity¹². The assay for ATPase activity was performed in 1.5 ml of 40 mM Tris-HCl (pH 7.5) with 6 mM ATP, 6 mM MgSO₄ and 0.2 mM CaCl₂ at $I = 0.12$ (adjusted with KCl) and at a myofibrillar concentration of 0.4–0.5 mg ml⁻¹. The reaction was terminated by addition of TCA and the Pi liberated was determined^{13,14}. Appropriate controls and reagent blanks were included in all experiments. Determinations of myofibrillar ATPase activity were made in triplicate at a series of temperatures between 0° and 18 °C. Activation energies (E_a) for the reactions over this temperature range were calculated from the corresponding Arrhenius plots. The Arrhenius plots were found to be linear within this temperature range for all species studied (statistical analyses given above). Thermodynamic parameters were calculated according to the following relationships¹⁵: $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$; $\Delta H^{\ddagger} = E_a - RT$; $\Delta S^{\ddagger} = 4.576(\log K - 10.753 - \log T + E_a/4.576T)$ and $K (s^{-1}) = V_{max}$ per mg of enzyme \times molecular weight $\times 10^{-3}$ mmol μmol⁻¹ $\times 1$ min per 60 s, where the molecular weight is expressed as mg mmol⁻¹ and V_{max} as μmol mg⁻¹ min⁻¹. The proportion of myosin in the myofibril was assumed to be 54% (ref. 7), with a molecular weight of 240,000 per enzyme site^{16,17}. All protein determinations were carried out using a Biuret method¹⁸.

*P = 0.01.
**P = 0.005.
***P = 0.001.

compensation is controversial¹. In the case of activation energy (E_a), as calculated from Arrhenius' equation, a correlation exists with habitat temperature for some enzymes^{2,5,6} but not others³. Studies of activation energy are principally concerned with the enthalpy of activation (ΔH[‡]). There have been comparatively few studies of the free energy of activation (ΔG[‡]) between homologous enzymes from animals of different thermal environments^{7,8}. Low *et al.*⁸ showed a correlation between ΔG[‡] for muscle type (M₄) lactate dehydrogenase and body temperature. The relative importance of enthalpic (ΔH[‡]) and entropic (ΔS[‡]) activation between poikilotherms and homeotherms was also shown to be different⁸. Similar results have been obtained for skeletal muscle myofibrillar ATPase activity⁷. Since these studies deal with homologous enzymes from animals with very different phylogenetic positions it is difficult to assess directly the adaptive significance of changes in the magnitude of these parameters.

We have determined thermodynamic activation parameters for the Mg²⁺-activated myofibrillar ATPase of the white muscle of teleost fish inhabiting a wide range of thermal environments from Antarctic to tropical waters.

A plot of log₁₀V_{max} against the reciprocal of the absolute temperature (K) is called an Arrhenius plot. Arrhenius plots for myofibrillar ATPase activity show a discontinuity at 18.5 °C (ref. 7). This effect has been attributed to the binding of Ca²⁺ to troponin A in systems containing the intact calcium-sensitising system, since it does not seem to be observed with pure myosin in the presence of high levels of Ca²⁺ or with desensitised actomyosin preparations⁹. In the case of some species of fish, myofibrillar ATPase activity also undergoes an initial activation before denaturation at higher temperatures^{6,10}. This initial activation effect makes accurate determinations of native specific activity very difficult¹¹. Although the occurrence of this initial activation varies considerably between species (occurring at lower temperatures in cold-adapted species⁹) it is not observed in any of the species studied at temperatures below 25 °C. To overcome these complications, we have considered only the temperature range 0–18 °C. The method of calculating the thermodynamic parameters,

ΔH[‡], ΔS[‡] and ΔG[‡] from the corresponding Arrhenius plots and V_{max} determinations is given in the legend to Table 1.

Values of ΔG[‡], the free energy of activation of the reaction, were broadly similar for all species studied. The values obtained for the Antarctic fish *Nothenia rossii*, however, were some 800 calorie mol⁻¹ lower than those of species living at the highest environmental temperature (Table 1). Small differences in ΔG[‡] between homeotherms and poikilotherms have been noted both for myofibrillar ATPase⁷ and M₄ type muscle lactate dehydrogenase, D-glyceraldehyde-3-phosphate dehydrogenase and muscle glycogen phosphorylase b⁸, and may be of some adaptive significance. Table 1 shows that a more important feature of temperature adaptation in this enzyme concerns the relative contributions of enthalpic and entropic activation. While ΔG[‡] was relatively constant between species the proportions of ΔH[‡] and ΔS[‡] varied considerably. A positive relationship between entropy of activation and environmental temperature was demonstrated. Values of ΔS[‡] varied from large negative values for the cold-adapted species to high positive values for the tropical species. Similar differences have been found between the entropy terms of the myofibrillar ATPase from birds and mammals where entropy was high and positive relative to amphibians and reptiles, where the values were low or negative⁷. There was also a strong positive correlation between enthalpy of activation and the mean annual habitat temperature of the species. Values for ΔH[‡] varied from 6,850 calorie mol⁻¹ for the Antarctic species (*Nothenia rossii*) (mean water temperature 0–2 °C) to 33,000 calorie mol⁻¹ for a species from an equatorial hot springs soda lake, *Tilapia grahami* (35–38 °C). Both enthalpies and entropies of activation were fairly independent of assay temperature.

A compensation plot¹⁹ of entropy change (ΔS[‡]) against enthalpy change (ΔH[‡]) for the different species was highly linear ($P < 0.001$) (Fig. 1). The slope of this plot has the dimensions of K and is called a proportionality constant or compensation temperature¹⁹. Our values of 280 and 300 K for assay temperatures 0.5 and 18 °C respectively are in the range reported for similar plots for a wide range of protein

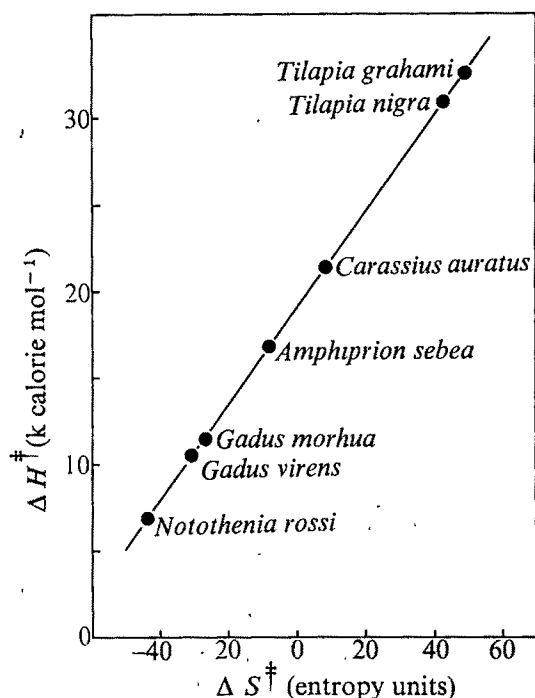


Fig. 1 The relationship between the enthalpy of activation and the entropy of activation for a range of species of fish living at different environmental temperatures.

reactions¹⁹⁻²². Examples include the binding of oxygen to vertebrate haemoglobin²⁰ and the formation of methaemoglobins for several mammalian species^{21,22}. The existence of a similar relationship between entropy change and enthalpy change, yielding similar compensation temperatures, for various processes of small solutes in water solution, has led some authors to implicate the participation of liquid water in these protein reactions^{19,20}. At present it is not clear whether the linear compensation patterns of these and other protein reactions are attributable to some general feature of protein-water interactions or arise entirely from some properties of the protein molecules themselves. If such compensation behaviour was found to indicate a general water-based phenomenon in protein reactions, this would have considerable biological importance in providing a theoretical framework both for the study of specific physiological processes and in the elucidation of mechanisms of molecular evolution.

Certainly, compensation behaviour of this type seems to provide biologically useful adjustments in the activation entropies and enthalpies for poikilothermic enzymes. The observed changes in ΔH^\ddagger and ΔS^\ddagger for myofibrillar ATPase, both of which increase from cold water to tropical habitats, are reflected in corresponding changes in the heat and entropy content of the muscle cells. Fish living at the higher environmental temperatures, where enthalpic activation is presumably facilitated, show a larger contribution of the enthalpic activation component. At low habitat temperatures, however, where enthalpic activation is likely to be energetically more unfavourable, the enthalpic component is partially replaced by a larger entropic contribution to the free energy of activation (Table 1). This has the advantage of greatly reducing the temperature sensitivity of the rate-limiting step in forming the activated enzyme-substrate complex. It might be expected that this would give significant adaptive advantage both to species which experience large fluctuations in environmental temperature and to those living in cold and temperate waters, since the limitations of the low heat content of the cellular environment have been partly offset.

It is known that myosins isolated from cold water species are characterised by a ready formation of aggregated products, within a few hours of preparation, and a concomitant decline of ATPase activity to zero²³. Such preparations are also more

easily denatured by urea and heat than the corresponding myosins from homoeotherms²⁴. It seems therefore that the concomitant structural adaptations in the tertiary structure of fish myosins consistent with their evolutionary modification for a low enthalpy environment necessarily make the molecule unstable at higher temperatures. Indeed a striking relationship has been shown between the thermostability of fish myofibrillar ATPase activity and the environmental temperature at which the species lives²⁵. The general order of thermal denaturation for this enzyme at 37 °C from 22 species of fish has been shown to be African equatorial lakes > Indian Ocean > Mediterranean > North Sea > Antarctic^{6,25}. The approximate half life of inactivation of the enzyme in comparable conditions varied some 350 times between these two temperature extremes. Species adapted to tropical environments presumably need a more rigid molecular structure to confer thermal stability at the higher environmental temperatures. Somewhat less marked correlations between thermostability and thermal environment have been shown for several mitochondrial enzymes in teleosts²⁶.

In view of the relevance of studies of myofibrillar ATPase to an understanding of muscle contraction, the molecular mechanisms of temperature compensation among this group of proteins seem to be of particular interest. A more detailed account of these phenomena will be presented later.

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C₄ photosynthesis in plants from cool temperate regions, with particular reference to *Spartina townsendii*

GREEN plants may be divided into two groups, C₃ and C₄ species, depending on whether the first product of photosynthetic CO₂ fixation is phosphoglycerate (C₃) or oxaloacetate (C₄). In all species studied to date the C₄ metabolism is associated

with either 'Kranz' leaf anatomy, low CO_2 compensation points or low $^{13}\text{C}/^{12}\text{C}$ ratios. Included in the C_4 category are many of the highest yielding and rapidly growing crops, most of which are believed to have originated in tropical and subtropical regions. C_4 species so far studied, which are mostly of tropical origin, attain their highest photosynthetic rates at leaf temperatures of about 30°C and above^{1,2} so that selective advantages derived from photosynthetic rate will only be evident in tropical and subtropical regions^{1,2}. A recent compilation of the reported rates of photosynthesis and productivity of C_3 and C_4 crops³ showed that differences in maximum annual dry matter yields could be explained by differences in lengths of growing season. It thus remains to be shown that there is a selective advantage which is a direct consequence of photosynthetic capacity of C_4 species or that there is an inherent physiological barrier to the adaptation of C_4 species to a similar range of latitudes to C_3 species.

There are two indirect consequences of C_4 photosynthesis which could be advantageous to plants irrespective of the climate in their region of origin. First, the ratio of weights of CO_2 assimilated to water transpired (the water-use efficiency) of C_4 plants, which is often twice that of C_3 species⁴, will be advantageous in habitats where water supply is limiting for growth or survival. Second, tolerance of salinity is a conspicuous attribute of many C_4 species. Indeed sodium is an essential micronutrient specific to C_4 species⁵ and may increase the *in vivo* activity of the primary carboxylating enzyme of C_4 photosynthesis, phos-

Fig. 1 Transverse section of a leaf of *Spartina townsendii* $\times 740$. The material was fixed in glutaraldehyde and post fixed in osmium tetroxide; after dehydration in ethanol, it was embedded in Epon and 1- μm sections cut for light microscopy²⁸. This section was stained with Toluidine blue and mounted in immersion oil. X, Bundle sheath cell containing chloroplasts. ('Kranz', the German word for wreath, was first used by Haberlandt⁷ to describe two concentric layers of chlorenchymatous tissues round the vascular bundles, the inner being the bundle sheath which contains chloroplasts, distinct from those of the outer mesophyll.)

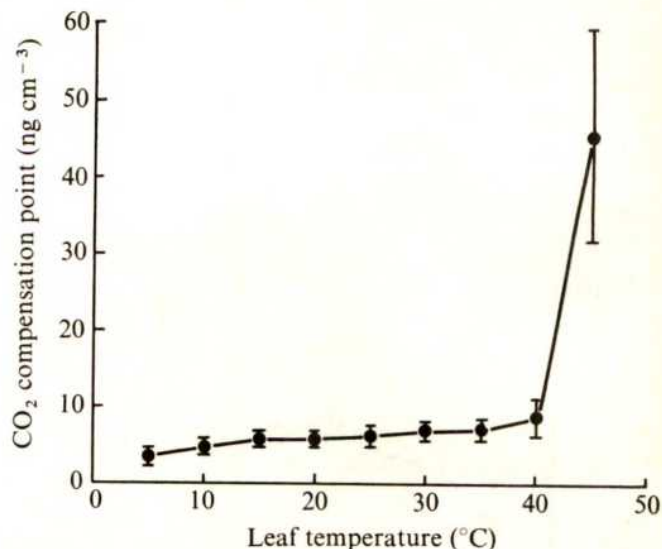
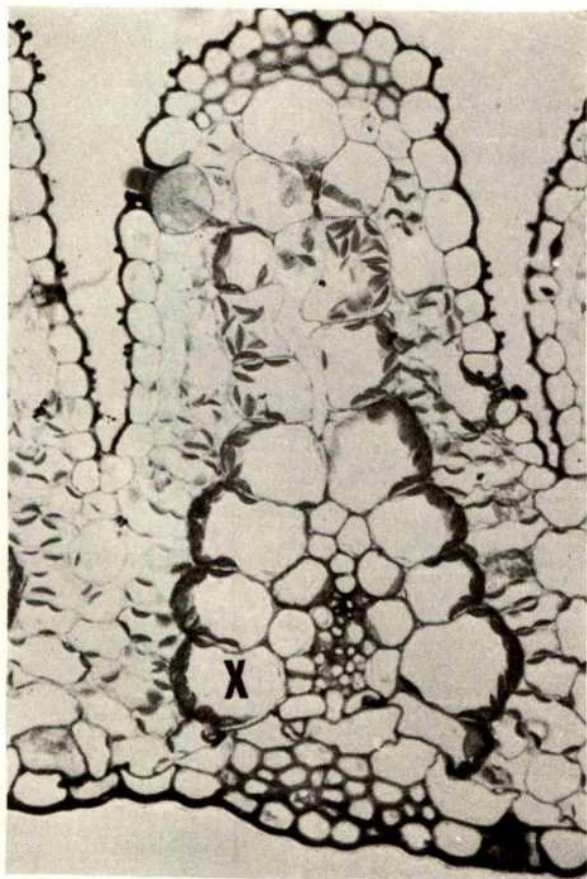


Fig. 2 Mean \pm s.e. of CO_2 compensation point for leaves of *Spartina townsendii* at nine leaf temperatures and photon flux density of about $200 \text{ nmol cm}^{-2} \text{ s}^{-1}$. The compensation points were determined using an infrared gas analyser (Analytical Development, Series 225) in a simple closed-circuit system. The analyser was calibrated in the range $0\text{--}100 \text{ ng cm}^{-3}$ using mixtures generated from pure N_2 and 1% CO_2 (Rank Precision Industries) with two gas mixing pumps (H. Wösthoff) in series. The concentration of the 1% standard was checked against mixtures generated from pure CO_2 and pure N_2 , and cross-checked by conductivity analysis²⁹. In practice it was found possible to discriminate differences of less than 0.2 ng cm^{-3} over this calibration range. Photon flux density at the leaf surface was determined using a Quantum sensor (Lambda Instruments)³⁰. Leaf temperature was determined with a copper-constantan thermocouple junction (diameter 0.1 mm) tightly appressed to the lower surface of the leaf.

phenol-pyruvate carboxylase⁶. We therefore searched for temperate C_4 species in physiologically dry and saline habitats.

Three out of thirty common higher plant species, collected from coastal sand dunes and salt marshes around the coast of Britain, were found to have the 'Kranz' leaf anatomy associated with C_4 photosynthesis: *Salsola kali* L., *Atriplex lacinata* L., and *Spartina townsendii* (*sensu lato*). *S. kali* has previously been noted to be a C_4 species⁸. *A. lacinata* and *S. kali* occur as far north as Sweden and are locally abundant at many sites around the British coast. *S. townsendii* is exceptional among the C_4 species now known to be native or introduced to Britain, in that it is a dominant component of large areas of our coastal salt marshes⁹.

Previous studies of *S. townsendii* have reported the bundle sheath to consist of large colourless cells^{10,11}, or only to contain chlorophyll in young plants¹². All material examined in populations from Southampton Water, the Ribble Estuary, and the Humber Estuary, however, showed 'Kranz' leaf anatomy (Fig. 1). In all cases the large bundle sheath cells contained numerous chloroplasts, often adjacent to the outer wall. Only the chloroplasts of the bundle sheath accumulated starch; they also differed in having twice the cross-sectional area and grana with fewer thylakoid stacks. All of these differences between chloroplasts have been found to a greater or lesser extent in the leaves of other C_4 species¹³, and clearly establish the occurrence of 'Kranz' anatomy in *S. townsendii*.

To test the anatomical inference that *S. townsendii* is a C_4 species we determined the CO_2 compensation point—that is the CO_2 concentration in air at which the net flux of CO_2 at the leaf surface in the light is zero. This is usually within the range $0\text{--}20 \text{ ng cm}^{-3}$ for C_4 species and $60\text{--}140 \text{ ng cm}^{-3}$ for C_3 species. The mean CO_2 compensation point for *S. townsendii* does not exceed 10 ng cm^{-3} over the range of leaf temperature $5\text{--}40^\circ\text{C}$ (Fig. 2) which confirms the occurrence of C_4 photosynthesis.

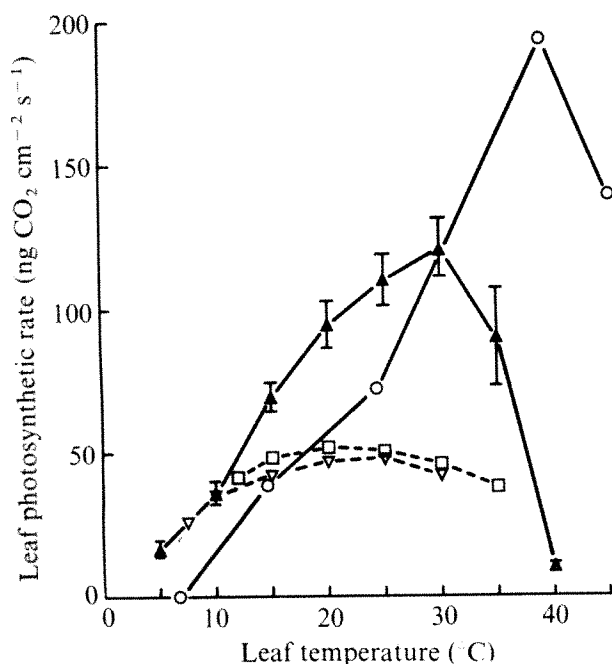


Fig. 3 Mean \pm s.e. of response of leaf photosynthetic rate at a photon flux density of about $200 \text{ nmol cm}^{-2} \text{ s}^{-1}$, to leaf temperature for *S. townsendii* (▲) compared with data obtained by others for three other grass species. Plants of *S. townsendii* collected from the Humber Estuary were grown in a controlled-environment cabinet (CM94PG, Fisons) at day-night air temperatures of 16/13 °C, a vapour pressure deficit of about 0.5 kPa and a photon flux density during the 16-h day of $50 \text{ nmol cm}^{-2} \text{ s}^{-1}$ at the crop surface. Nutrient solution (200 ml) (Solu-feed, ICI) was added to each 15-cm diameter plant pot once every 3 weeks. Steady-state leaf photosynthetic rate was determined in an open-circuit gas exchange system. A glass water-jacketed, air-sealed, leaf chamber was used to provide accurate environmental control over single attached leaves. The light source was a high pressure sodium lamp (SON/T 400W, Philips) with a parabolic reflector. Photon flux density and leaf temperature were measured as described in Fig. 2. Ambient CO_2 concentration in the air supplied to the leaf was maintained at 600 ng cm^{-3} , and the water vapour pressure deficit at 0.3 kPa. The net flux of CO_2 at the leaf was determined by differential gas analysis. The infrared gas analyser was calibrated by the split-tube method of Parkinson and Legg³¹ using gas mixtures generated by the method described in Fig. 2. Growth of material and measurement of photosynthesis and microclimate for *Sesleria albicans* (▽; ref. 15), *Festuca arundinacea* cv. S170 (□; ref. 16) and *Pennisetum purpureum* cv. 05088 (○; ref. 14) was by similar methods to those described here.

Although many C_4 species may attain leaf photosynthetic rates often three times those of C_3 species, these differences are only realised at relatively high temperatures. The C_4 species, *Pennisetum purpureum*, is reputed³ to be the world's highest yielding forage crop. At 38 °C its rate of leaf photosynthesis¹⁴ is some three times the maximum obtained by two temperate C_3 grasses *Sesleria albicans*¹⁵ and *Festuca arundinacea*¹⁶ (Fig. 3). At 16 °C, however, the mean air temperature for the warmest month in South-east Britain, its rate is lower than that obtained by the C_3 grasses. In common with other C_4 grasses, photosynthesis in *P. purpureum* ceases at leaf temperatures of 7 °C and below, whereas *S. townsendii* maintains significant photosynthetic rates at 5 °C (Fig. 3) and rates at relatively low temperatures, 5–10 °C, comparable with C_3 grasses (Fig. 3). The leaf photosynthetic rate of *S. townsendii* at 25 °C is about 50% higher than the maximum rates reported for the major herbage grasses of Britain¹⁷. Its temperature optimum for photosynthesis (30 °C) however, is 7–11° lower than optima for other C_4 grasses, measured in similar conditions^{14,18,19}. Net

photosynthetic rate in *S. townsendii* declines with increase in temperature above 30 °C, so that at 40 °C it is 10% of the rate at 30 °C. This does not result from the occurrence of photorespiration at these temperatures, because the compensation points from 30–40 °C do not differ significantly from those at lower leaf temperatures (Fig. 2).

S. townsendii is considered to have arisen from the hybridisation of *S. maritima* (Curt.) Fernald, endemic to Europe and Africa, and *S. alterniflora* Lois., a species introduced from North America²⁰. *S. alterniflora* is one of five species of the genus *Spartina* native to North America which have previously been suggested to be C_4 (refs 21–25). *S. maritima* (Mersea Island, England) possessed typical 'Kranz' leaf anatomy. Since its first recorded appearance at Southampton Water in 1870, *S. townsendii* has spread, both by natural dispersal and by deliberate plantings, to occupy over 12,000 hectares of intertidal mudflats around the British Isles. Attempts to introduce *S. townsendii* to latitudes below 48 °N have repeatedly failed, but it has been successfully introduced to Udale Bay, Scotland (57.5 °N), and to salt marshes in Denmark where it now occupies over 500 hectares (ref. 26). This pattern of distribution suggests that *S. townsendii* is a plant limited to cool temperate regions and thus affords an unrivalled opportunity to examine the potential of C_4 photosynthesis in these conditions.

It is of interest that the perennial *Spartina gracilis* Trin., an inland North American species, which has a distribution extending to above 60 °N into the North West Territories of Canada²⁷, also has 'Kranz' leaf anatomy.

In conclusion, *S. townsendii* is a C_4 species adapted and limited in distribution to cool temperate climatic regions. One feature of this adaptation is the ability of the species to maintain significant rates of photosynthesis at relatively low temperatures, which contrasts with observations on other C_4 grasses. Thus, C_4 photosynthesis is not inherently limited to subtropical and tropical regions and physiological adaptation of this process to a cool temperate habitat has occurred.

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X-ray evidence of temperature-dependent conformational disorder of hydrogen-bonded carboxylic acid molecules in crystal structures

FROM an analysis of X-ray data on carboxylic acids¹ we have established the stability of the approximately coplanar arrangement of the non-hydrogen atoms of the acetic acid group (C-COOH) with the α substituent in α -substituted carboxylic acids (Fig. 1). In the crystal structures of tartaric acid (X = OH) (ref. 2), aminomalonic acid (X = NH₂) (ref. 3) and fluoroacetic acid (X = F) (ref. 4) the coplanar *cis* conformation has been found. In crystals of unsubstituted monocarboxylic acids the acetic acid group has been found to be coplanar with the β -carbon atom¹.

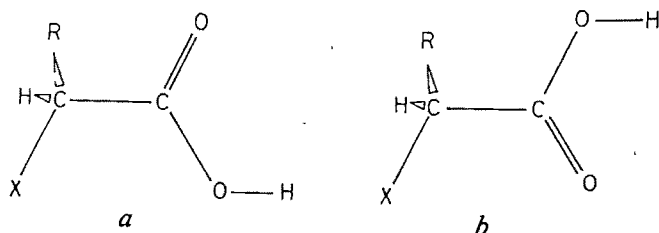


Fig. 1 *Trans* (a) and *cis* conformation (b) in α -substituted carboxylic acids.

From infrared studies of unsubstituted monocarboxylic acids at different temperatures, Hayashi *et al.*^{5,6} deduced that in odd-numbered acids the crystal contains the *cis* form at lower temperatures, whereas (lower members excepted) crystals of the even-numbered acids contain the *trans* form. As yet, X-ray evidence to support these conclusions is scarce⁷⁻⁹. Hayashi also pointed out that by assuming the difference in energy between the *cis* and *trans* forms to be small, it may be expected that they coexist in crystals of carboxylic acids in various temperature-dependent statistical proportions.

A feature common to the crystal structures of carboxylic acids is that the carboxyl groups predominantly couple with one another to form the well known eight-membered rings. A transition from the *trans* to the *cis* form or *vice versa* may be conceived of as a simultaneous transport of the protons along the hydrogen bond, accompanied by a rearrangement of the electron density and subsequent changes of bond geometry in the various carbon-oxygen bonds, packing considerations ruling out the mechanism involving a rotation of the carboxyl groups (Fig. 2). The assumed coexistence of the *trans* conformation and that of *cis* in different ratios in crystals will imply that in the X-ray analysis C-O bond lengths and C-C-O bond angles are found that are weighted averages of C=O and C-O lengths and C-C-O and C-C=O angles, respectively.

This variation in the carboxyl group geometry is, as far as bond lengths go, subject to the Speakman rule¹⁰, which states that the sum of the carbon-oxygen distances will be 2.52 ± 0.02 Å. For example, at room temperature (RT) C-O bond lengths in benzoic acid¹¹ are 1.24 and 1.29 Å, whereas in tartaric acid² these distances are 1.21 and 1.30 Å.

It is in the RT structure of fluoromalonic acid¹², where C-O lengths and C-C-O angles are equal within experimental error (Table 1), that this supposed *cis-trans* disordering must be at its maximum. The expectation that such a disorder would split

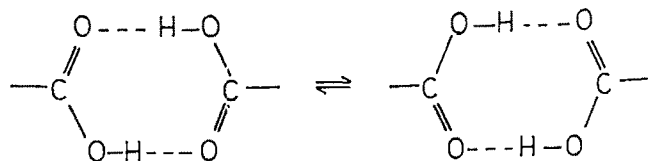


Fig. 2 *Cis-trans* transition by proton transfer in a cyclic carboxylic acid dimer configuration.

the hydrogen atom electron density along the hydrogen bond donor-acceptor line, we found to be true in a subsequent, more accurate redetermination of the crystal structure of fluoromalonic acid (Fig. 3a). Hayashi's concept of temperature-dependent disordering in crystals of organic acids prompted us to look into the change in carboxyl group geometry at liquid nitrogen temperature (LNT) in crystalline fluoromalonic acid. As this acid constitutes the ideal test case, a temperature decrease here dictates a drastic alteration of disordering.

X-ray results derived from data obtained from a single crystal of the highly hygroscopic acid, prepared by sublimation *in vacuo*, are summarised in Table 1. Cooling selectively reduces the length of the *b* axis by about 0.4 Å, whereas the *c* axis increases by about 0.09 Å. The carboxyl group geometry tends to be similar to that of a 'normal' carboxyl group. The hydrogen atom electron density splitting has disappeared and

Fig. 3 Electron density difference synthesis in the plane of the carboxyl group dimer. Contours on an arbitrary scale. a, RT; b, LNT.

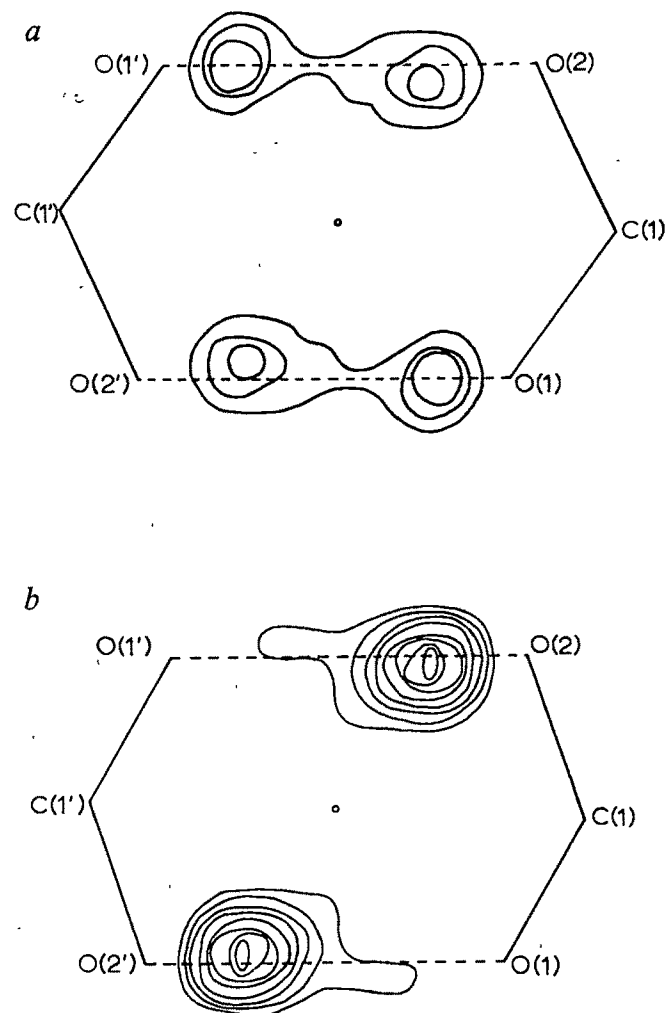


Table 1 Summary of X-ray crystal structure analysis of fluoromalonic acid at RT and LNT

	RT	LNT
Cell dimensions	$a = 4.593 \text{ \AA}$ $b = 8.322$ $c = 11.214$	4.568 \AA 7.930 11.307
Radiation	Cu(K α)	Cu(K α)
Number of reflections	415	398
Space group	Pnam ($Z = 4$)	Pnam ($Z = 4$)
R factor	0.032	0.042
$R = \frac{\sum F_o - F_c }{\sum F_o }$		
C(1)–O(1)	1.251(2) \AA	1.224(2) \AA
C(1)–O(2)	1.254(2)	1.291(2)
C(2)–C(1)–O(1)	116.5(2)°	119.5(2)°
C(2)–C(1)–O(2)	117.1(2)	114.1(2)
O(1)–C(1)–O(2)	126.4(2)	126.4(2)
C(1)–C(2)	1.523(2) \AA	1.529(2) \AA
C(2)–F	1.367(3)	1.373(4)
C(2)–H(1)	0.96(3)	0.95(4)
O(2)–H(2)	Disordered	0.88(3)
C(1)–C(2)–C(1')	111.4(2)°	111.1(2)°
C(1)–C(2)–F	110.1(1)	110.5(2)
C(1)–C(2)–H(1)	108(1)	107(1)
H(1)–C(2)–F	109(2)	109(2)
C(1)–O(2)–H(2)	Disordered	105(2)
Hydrogen bond: O(1)–O(2')	2.657(2) \AA	2.649(2) \AA
('') = 1 – x, – y, – z.		

an asymmetric single maximum is now found in the electron-density difference map (Fig. 3b). As the molecule at LNT clearly has the *trans* form, the question arises as to whether the established *cis* stability at RT in crystals of α -hydroxyacids and of fluoroacetic acid also applies to these structures at LNT.

The results for fluoromalonic acid give the first unambiguous X-ray evidence of conformational disorder at elevated temperatures in molecular crystals of carboxylic acids. Note that the RT X-ray analysis of the crystal in question after the LNT experiment again shows the same geometrical anomalies, which can now definitely be ascribed to conformational disorder.

We thank Dr Hayashi for making available his results before publication.

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Errata

In the article "The modulation contrast microscope" by R. Hoffmann and L. Gross (*Nature*, **254**, 586, 1975) equation (1) should have read

$$U_{(0)} \sim \int e^{-i\phi x} e^{-i\theta x} dx$$

and not as printed.

In the article "Localisation of human peptidase-A structural locus from studies on a cultured lymphoblastoid line" by E. Arthur, C. M. Steel, H. J. Evans, S. Povey, B. Watson and H. Harris (*Nature*, **257**, 308; 1975) the legends for Figs 2 and 3 were transposed. The figures are correct as they stand.

Corrigendum

In the article "A resonant point absorber of ocean-wave power" by K. Budal and J. Falnes (*Nature*, **256**, 478; 1975) the absorption length $d_a = P_{\text{opt}}/K$ was estimated to be $d_a = 50$ m in small-amplitude sinusoidal waves and $d_a = 20$ m in real North Atlantic waves when the tank diameter is $2a = 16$ m. This result was based on the approximation that diffraction effects are negligible when $a \ll \lambda$ where λ is the wavelength. Unfortunately, this approximation overestimates d_a when a is as large as 8 m. Including diffraction effects in the calculation it is found that the absorption length in small-amplitude sinusoidal waves is $d_a = \lambda/2\pi$, irrespective of the value of a . This result is valid for any circularly symmetrical, optimised, resonance-tuned heaving body on deep water. In small-amplitude sinusoidal waves of period $T = 10$ s this gives $d_a = 25$ m instead of $d_a = 50$ m. In the case of wind-generated waves the overestimation of d_a is less drastic. The general conclusions drawn regarding the advantage of point absorbers are still valid.

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reviews

OVER the last decade the quest for a semantics of natural language has reached the proportions of an intellectual's crusade. Dedicated regiments of linguists, logicians, psychologists, and computer scientists, have set off for the Holy Land, and massive amounts of intelligence, both real and artificial, have been directed at what turns out to be a most intractable infidel. There have been a few piecemeal victories, but the campaign so far has been a failure. There is no single hypothesis that provides a convincing and general theory of what meaning is.

Walter Kintsch's book offers us a psychologist's view of the battleground, and it illustrates many of the strengths and most of the weaknesses of this perspective. Kintsch believes that the essential strategy is to find the right notation. He represents the meaning of a sentence such as, "Peter put the book on the table", by the formula: (PUT, AGENT: PETER, OBJECT: BOOK, GOAL: TABLE).

That is all very well, but what is the notation for; what does it do? Kintsch does not tell us. Nor does he tell us precisely what he means by AGENT, OBJECT, or GOAL. Indeed, one of the great drawbacks of these notions, which derive from Fillmore's 'case' grammar, is that no one has ever succeeded in stipulating a meaning for them that is wholly explicit and that requires no exercise of intuition on the part of the reader. The trouble with doing semantics by translating an expression from one language into another is that one needs a semantics for the 'meta'-language. Kintsch does not provide one, and so it is singularly unsatisfying to learn that the meaning of 'put' is 'PUT'. But at least at this point we reach a current controversy.

GOAL: MEANING

P. N. Johnson-Laird

The Representation of Meaning in Memory. (The Experimental Psychology Series.) By Walter Kintsch. Pp. vii+279. (Lawrence Erlbaum: Hillsdale, New Jersey, 1974.) £8.00.

Everyone agrees that the meanings of many words can be decomposed into more basic constituents: 'kill' can be analysed, for example, as 'cause to become not alive'. The question is: should this analysis be part of the meaning of the word as it is represented in the mental dictionary, or should it be captured by rules ('meaning postulates' as Carnap originally dubbed them in another context) for interrelating different expressions in the language? In a variety of experimental manipulations, Kintsch failed to find any evidence for an actual decomposition into more primitive elements, and he accordingly rejects the notion of analytical dictionary entries in favour of meaning postulates. There seems to be little reason to accept this conclusion.

Analytical entries may well exist, but may only be consulted in certain circumstances as, for example, when a speaker attempts to define a word, or a listener attempts to verify a sentence. Indeed, it is hard to know what empirical data would in principle decide the issue. A further problem with rules which merely relate expressions is, of course, that they provide no way of relating language to the world, no way of determining the truth or falsity of an assertion. Kintsch's theory does not

even attempt this task, not does it offer any systematic account of meaning postulates. It is a semantics without any semantics.

The one idea that does emerge from the theory is that sentences, or at least assertions, express underlying propositions. A sentence such as, "The fire destroyed many acres of virgin forest", contains four underlying propositions: (DESTROY, FIRE, FOREST); (SIZE, FOREST, ACRE); (VIRGIN, FOREST); and (MANY, ACRE). The notation presents numerous logical problems, but fortunately nothing crucial hangs upon it. Kintsch is able to predict a variety of aspects of the processing of prose simply on the basis of the number of propositions, and their gross interrelationships, that are expressed in it.

The content of the book changes abruptly in calibre in its second half, which is devoted largely to an account of a series of original experiments concerning the reading, representation, and recall, of paragraphs. This research is a sustained and sometimes ingenious attack on an intriguing psychological problem.

One final warning. This book is suitable reading only for psychologists. Logicians will be repelled by its logical inadequacies. Linguists will find little new in it. Computer scientists will be deterred by its failure to grapple with the function of a semantic theory. Psychologists, however, will find in it some stimulating experiments, written up in the detailed style of journal articles. They may also gawp at the mathematical filigree—flashy, but perhaps irrelevant—with which the author fits model to data. Meanwhile, back in the Holy Land, Jerusalem remains unconquered. □

THERE are many books on bees but few on the end product of their labours. Most books on honey are in the realms of folk law and health foods. As a result this text by a distinguished group of international specialists backed by the resources of the Bee Research Association is a landmark.

Divided into five sections with sixteen chapters the content has a potential appeal to a wide readership. Sections 1 and 3 dealing with the production and preparation of honey will appeal to beekeepers. Section 4 deals with the commercial aspects and will interest those involved in international trade and legislation.

Section 2 is the major scientific contribution covering the chemical, physical and biological characteristics and is the first attempt to put the

Honey for some

Paul Wix

Honey: A Comprehensive Survey. Edited by Eva Crane. Pp. xvi+608. (Heinemann: London, 1975.) £15.00.

material into a book where it can relate to wider issues. Section 5 has its appeal for those interested in the history and language associated with honey.

The scientists will find detailed

treatments of the chemical, physical and microscopic (pollen analysis) data, and under the brief section on biological properties every attempt has been made to separate fact from folk law. This represents a starting point for those who consider that honey still holds most of its biological secrets close-hidden.

The late 1600s woodcut with the Latin "So we the bees make honey, but not for ourselves" is a fitting tribute to the long association between man and bees. At £15 it is fairly priced although as a book of reference it might have been wise to have increased the price in favour of a paper with a finer finish. □

The Filament Fungi, Volume 1, *Industrial Mycology*. Edited by J. E. Smith and D. R. Berry. Pp. xii+340. (Edward Arnold: London, April 1975.) £12.50.

THIS is the first volume of a series devoted to the filamentous fungi and is intended to be used by senior undergraduate and postgraduate students as well as by workers involved in the pharmaceutical, chemical and food industries. The industrial applications of yeasts have been fully considered elsewhere and have not been included.

Twenty authors have contributed the sixteen chapters which constitute this volume. The backgrounds of these authors are somewhat different but it is of interest that all except four are at present working in Britain. Academics are more frequently represented than those engaged in work in industrial establishments.

No attempt is made to provide any treatment of the taxonomy of filamentous fungi or to provide assistance in their recognition. The first portion of the book deals with such fundamental aspects as structure and development, environmental control of physiology, secondary metabolism and its relationship to growth and development, strain improvement and stability and growth kinetics. The fungi are particularly prolific in the production of a wide range of secondary metabolites and since only a minority of species has so far been studied the real extent of the range of products they can produce is likely to be shown to be very much wider than is at present appreciated.

The second portion of the book deals mainly with the fungal fermentation industry. In an account of the historical development of this industry it is shown that the discovery of penicillin, followed by other antibiotics, provided the main initial impetus for the rapid expansion of the industry at a time when the background knowledge made such an expansion possible. After a discussion of commercially important secondary metabolites there are chapters dealing with the commercial production of organic acids, the transformation of organic compounds by fungus spores, industrial enzyme production, industrial exploitation of ergot fungi and oriental food fermentations. In a chapter entitled 'Submerged culture production of mycelial biomass' the modern developments in the production of protein by mycelial biomass and in the production of mycelium for use as flavouring agents are discussed. Three useful chapters on the cultivation of edible mushrooms, mycotoxins and biodeterioration and biodegradation by fungi are provided.

Not only does the text provide an up-

to-date and comprehensive treatment of the subject but the citation of a considerable number of publications at the end of each chapter is useful. The book is well produced although the few photographs are rather poorly reproduced. It represents a valuable review of an important field of study.

J. Colhoun

Mycogenetics: An Introduction to the General Genetics of Fungi. By J. H. Burnett. Pp. xiv+375. (Wiley: London and New York, 1975.) £12.50, cloth; £6.00, paper.

THIS work gives wide coverage to all the significant aspects of fungal genetics, and comprises four sections: structure and life histories of the various groups of fungi; formal genetics (genetic markers, linkage and recombination in

Fungi

meiotic and mitotic systems, extra-chromosomal elements and quantitative inheritance); population genetics (variation, selection and isolating mechanisms); industrial applications; genetics of fungal pathogenicity; and the mechanism of recombination and of gene action and regulation in fungi. Although there are no photographic plates, the text is admirably illustrated with numerous diagrams, figures, line drawings, tables and graphs. Worked examples are included and should prove valuable in understanding the complexities of, for example, the analysis of unordered tetrads and of certain aspects of quantitative inheritance.

The text is not easily read because of the extreme condensation of research publications, the profusion of technical terms and frequent directions to refer backwards or forwards to chapters or illustrations. There is also the interpolation of numerous references to original work. The introductory reader must be prepared to cope with the 'jargon' liberally sprinkling the text, as no glossary of these perplexing terms is given. Indeed, a few of the terms are not even defined in the text: for example, introgression, allopatric and sympatric.

The author must be taken to task on a number of points where I feel important omissions, ambiguities or even errors have been made. For instance, in the legend to Figure 5.4 it is mentioned that 43 out of 120 *trp-1* mutants of *Neurospora crassa* show no complementation with each other or with any of the mutants which fall into the eight complementation groups. The significance of such non-complementing mutants is not considered.

In the analysis of ordered tetrads, the

author states that the centromere distance of a locus is half the percentage frequency of second-division segregation asci for that locus because "a single cross over involves two of the four chromatids, that is, half of them". In fact, in higher diploid organisms the map unit was based directly on recombination percentage which represents the percentage frequency of "half chiasmata". In a second-division ascus, a whole reciprocal chiasma has been detected and so the percentage frequency of second-division asci is divided by two to give the centromere distance in conventional map units. The author then states that double cross overs between the locus and the centromere will be recorded as first-division segregations. This is not true because a three-strand double cross over will be recorded as a second-division ascus, as if a single cross over had occurred. The author attributes this "reduction in the real incidence of recorded cross over" to account for a maximum value of 67% second-division asci. This statement is somewhat ambiguous.

In the explanation of hybrid DNA formation during recombination between two of the four chromatids with the resulting formation of mis-matched base pairs, the author states, "Any mis-matched base pairs in the 'hybrid DNA' region are then corrected". He fails to realise that sometimes none of the mis-matched base pairs may be corrected so that no gene conversion occurs but post-meiotic segregation leads to the formation of "aberrant 4+:4m asci", with 'misplaced' spores, but in which the outside markers show normal 4+:4m segregations. Such asci are not mentioned yet they provided the key to the understanding of gene conversion. Consequently the author is, as it were, one step out in the subsequent possibilities.

Finally, the author does not mention valuable work and models proposed by Professor Olive and coworkers or the still more recent work by various researchers.

Those aspects of fungal genetics with which the author is familiar and to which he has contributed by original research, are treated in a full, interesting and up-to-date manner. To be fair to the author, he does state in the preface that he has tried to concentrate on general issues rather than to report the latest findings on any particular topic, but this method is likely to result in uneven treatment of the various aspects. He tells us also in the preface that the book is intended not only for geneticists but also for mycologists, pathologists, industrial users of fungi and biologists. I fear, however, that most of the latter types of reader will find the text turgid and tough going.

L. C. Frost

Computer checks . . .

Computer Chess. (ACM Monograph Series.) By Monroe Newborn. Pp. xii+200. (Academic: New York and London, April 1975.) \$15.00; £7.20.

1975 SEES the 25th anniversary of Claude Shannon's *Programming a Digital Computer for Playing Chess* (*Phil. Mag.*, 41, 356; 1950), and it is an appropriate time for a review of the developments which have occurred in this field since the appearance of that classical paper.

The better computer programs in the US and USSR are now achieving standards of play which make them interesting, not only to the computer scientist, but also to the chessplayer, for, in the games of computers, the chessplayer can recognise patterns of play which are familiar among human chessplayers. Indeed, for me, a principal fascination of this book arises from the existence in computer games of traits which I would, in human players, regard as personal styles, and yet which are, in the computer, seen to be consequences of simple arithmetical processes. This is not to imply, however, that the computers have yet reached human capability, even on the chessboard, for the absence of that indefinable human quality known as 'common sense' is all too evident in the computers' deliberations. That absence is perhaps the outstanding feature of all computer chess, and makes it unmistakably different from the human variety, for, unlike the human player of comparable ability, the computer will often engage in study of local minutiae, while obvious catastrophe threatens in another sector. Examples of this amusing behaviour are frequent in the book, and will reassure the reader who fears that his mind may soon be superseded.

The full appreciation of this book requires from the reader a moderate knowledge of chess. Fortunately, there is no corresponding requirement regarding a familiarity with computer programs. Thus, in my view, the other principal success of the book lies in its presentation of the structure and mechanics of computer chess programs in a manner which is not subject to obscurity by computer software jargon. Using simple language, the author presents a short, general survey of the techniques common to most chessplaying systems, and he includes a more detailed description of one of his own, quite successful, programs.

The central part of the book consists of some 36 analysed computer games. In the analysis, both the situation as interpreted by a competent chessplayer, and the rather different interpretations made by the computers, are presented in parallel as the games proceed. The games are taken principally from American intercomputer tournaments,

and cover the period 1967-74, thus enabling the reader to assess the progress, up to quite recent times, of this rapidly evolving art.

Overall, I would say that this book represents a successful navigation of the straits between the Charybdis of obsolete tedium and the Scylla of trivial immediacy which flank this rapidly developing subject.

Nigel J. Holloway

. . . into the past

Mathematics and Computers in Archaeology. By J. E. Doran and F. R. Hodson. Pp. xi+381. (Edinburgh University Press: Edinburgh, 1975). n.p.

FEW, if any, archaeologists now hold the view that 'science' has no place in archaeology, and in recent years mathematics and computing have become increasingly recognised as indispensable archaeological aids. The authors of this book rightly criticise some previous workers who have attempted to become 'scientific' by seizing on fashionable theories and forcing archaeological data into them, with a concomitant naïve and empirical use of borrowed 'hypothetico-deductive' jargon. This is an eminently readable book, refreshingly free from such jargon, which is intended for the archaeologist with no specialised mathematical knowledge, and which serves as an introduction to the subject, a critical summary of previous work, and an invaluable reference manual. It is written with simplicity but without condescension, the style being marred only by frequent confusion over the use of the first and third person and by an unnecessary proliferation of exclamation marks.

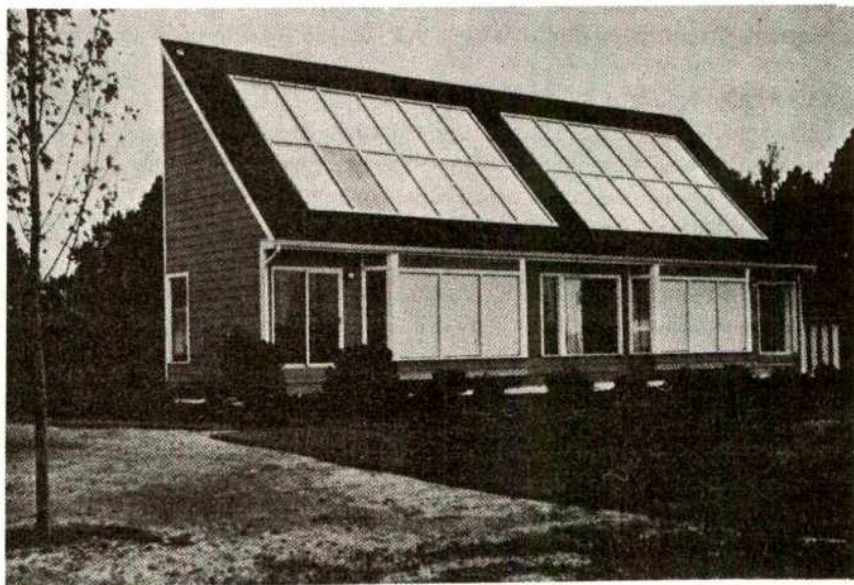
In Part 1, which deals with basic archaeological and mathematical tools,

care is taken to explain fundamentals such as algorithms, vectors and functions, in terms quite comprehensible to the intelligent innumerate. Simple examples are chosen to illustrate such concepts as probability, and there is an excellent section on sampling and significance testing.

Part 2, which is devoted to data analysis techniques, presents a number of ways of dealing with the regularly recurring archaeological problem of classifying great masses of highly complex items. Reasoned, if caustic, arguments are levelled against much of the mythology of the 'New Archaeology', and at the many invalid approaches to taxonomy which have been used in the past with only partial understanding of the numerical methods involved. The section closes with a useful series of examples of data analyses, including Hodson's comparative taxonomy and seriation of the Münsingen La Tenè fibulae.

Part 3, which is concerned with problems and prospects for the future, includes especially helpful sections on automatic seriation techniques and the use of computer-based archaeological data banks.

This is a volume to be thoroughly recommended, both as an introduction to first principles and as a practical comparison of the different techniques, including an assessment of their theoretical value. The general presentation of the book is of a high standard, but suffers from occasional erratic spacing and printing, together with poor reproduction of some of the simpler sketch-diagrams. At £8.00 for nearly 400 pages it is expensive but not overpriced, and it will undoubtedly remain the standard introduction to the subject for several years to come. M. L. Shackley



Solar one, a solar electric home built by the University of Delaware. From *Energy Resources*. By Andrew L. Simon. Pp. x+165. (Pergamon: Oxford and New York, 1975.) £6.40, \$15.00, boards; £3.40, \$8.00, paper.

Synaptology

Synapses and Synaptosomes: Morphological aspects. By D. G. Jones. Pp. xiv+258. (Chapman and Hall: London, July 1975; distributed in the US by Halstead.) £14.40.

THE demonstration by Whittaker and by De Robertis in the early sixties that nerve terminals carrying synaptic complexes can be isolated in enriched fractions after controlled tissue rupture, initiated a new approach for synaptology and caused an urgent reappraisal of the nature and purity of subcellular fractions from brain tissue. Gareth Jones' monograph sets out to provide an up-to-date review of the current picture of knowledge on nerve terminals, synaptic complexes and their *in vitro* derivatives, the synaptosomes. Although the subtitle predicts a basically ultrastructural content, the text ignores this constraint and provides a biological overview of the synapse, dealing with the biochemical and physiological correlates of the observed morphology.

The final chapter gives in outline the continuing debate over the source of transmitter released in to the synaptic cleft, and deals with the vesicle hypothesis, exocytosis, and the current indications that contractile proteins may be involved in the mechanism. Another chapter describes in some detail the preparation of synaptosomes from the precise conditions of tissue rupture, the use of gradients of different types and compositions, and subfractionation of the synaptosome itself. The use of synaptosome-enriched fractions in the study of nerve terminal metabolism and of transmitter synthesis, storage and release constitutes a separate chapter. In particular it emphasises the awakening of neurochemists to the opportunities offered by the fact that synaptosomes are sealed structures, most likely generating membrane potentials and carrying a full complement of metabolic and transmitter-linked enzyme systems, fully capable of functioning in a controlled and integrated fashion for many hours *in vitro*. Moreover, they respond to depolarising stimuli with transmitter release.

The real strength and authority of the book, however, is in the two chapters on ultrastructure, reflecting the author's long association with synaptic morphology. He deals with the synapse *in situ* and the changes which may be seen in the derived synaptosome. There is a useful account of differential electron microscopic staining techniques for various synaptic constituents and components, using phosphotungstic acid, bismuth-

iodide-uranyl acetate or zinc iodide-osmium tetroxide mixtures. There is an appraisal of the ultrastructural status of an array of synaptic components including pre- and postsynaptic dense projections, coated (complex) vesicles, baskets, cytonets, reticulosomes, tetrasomes and other structures observed using new fixation, staining and other visualising methods.

The text is written in an interesting and vigorous style and is fully illustrated with copious line drawings and over 100 micrographs. It is a useful addition to the literature on synaptology and will no doubt find its way into most libraries of neurobiology provided the price is not too strong a deterrent.

H. F. Bradford

Chordate morphology

The Chordates. By R. McNeill Alexander. Pp.vi+480 (Cambridge University, April 1975.) Cloth £10.00 \$28.50; paper £4.25.

MORPHOLOGY is a dull subject, and there are only two good reasons for teaching it: either that the structures are important in the animal's way of life, or that they illuminate its evolution and relationships. But, in many universities, the systematic morphology section of zoology is now isolated in a separate course unit, divorced from the related physiological and ecological viewpoints which help to make it significant and interesting.

Those who teach such courses therefore have to find individual topics in which the importance of morphology is shown clearly, or in which its study can be used to exemplify the aims and methods of modern research. The adaptive significance of many of the gross morphological structures is clearly obvious, and is already described in many textbooks. But analyses of the mechanics of swimming or mastication, for example, have developed a great deal since most of the commonly-used textbooks of vertebrate biology were written. Alexander's book not only provides these up-to-date analyses, but also a similar range of examples of the ways in which vertebrate structures are physiologically adapted. He has succinctly set out his aims in the Preface:

"Most of the book is about animal structures, how they seem to have evolved and how they work. A great many experiments are described because it is often as interesting and worthwhile to know how an item of knowledge was obtained as to know the item itself. Since the book is about structures, many of the explanations involve physics or engineering. There are many simple calculations designed either to throw light on the dimensions of a structure, or to test the plausibility of an explanation.

There is information in this book about locomotion, respiration, blood circulation, feeding, osmotic and ionic regulation and sense organs. These topics relate well to gross structure."

Alexander has cast his net fairly widely. For example, some of the topics covered in the first 200 pages of the book are rates of water flow through gills and of gas diffusion into gills, the structure and mechanical properties of bone, the hydrodynamics of swimming, the mechanics of jaw and gill action, the physics and physiology of teleost swim-bladders, the optics of silvery reflective fish camouflage and the physiology of electric organs. Such topics are considered thoroughly and clearly, as relevant to the life of the group in question. They are dealt with at both the theoretical and the experimental level, and will help students to realise that morphology is really only a series of problems in adaptation, not all of which are, as yet, properly understood.

The book does not pretend to uniform coverage of the different chordate groups; 190 pages are devoted to fish, as against 230 for the remaining vertebrates. Criticism of this imbalance could fairly be countered by pointing out that it does not matter which group is used to illustrate a principle, as long as it is adequately illustrated. Also, since Alexander's research has been centred on fish, it is natural that he should turn to them for many of his examples. Nevertheless, it does mean that those of us whose sympathies have accompanied the tetrapods onto land will feel a little disappointed.

About half of the 214 figures were drawn especially for the book, and many of the remainder have been taken from research papers. Many will be new to those accustomed to more conventional textbooks of vertebrate morphology, for there are many figures of experimental apparatus, graphs and physiological recordings of different kinds. The diversity of sources has led to a variety of styles, and a few figures have reproduced poorly.

This book does provide a new and up-to-date functional approach and it is a valuable addition to the library of anyone designing a course in vertebrate zoology, or for students taking such a course (for whom there is a reasonably-priced paperback edition). Of course, this new approach has been at the expense of the conventional coverage of such topics as the adaptive radiation of the groups, or their relationships to one another or to geological periods and time. But for those of us who want it, this information is easily available in other books, and such omissions are a small price to pay for such a welcome, innovative book.

Barry Cox

nature

October 23, 1975

Are scientists really stifling science?

SCIENTISTS are stifling science; Professor John Taylor of King's College, London put this proposition to an audience at the Royal Institution in a televised "Controversy" programme this week. Within the necessary confines of a televised debate it is all too easy to judge the matter won or lost on style rather than content; Professor Taylor raises an important issue and it merits careful consideration within the scientific community.

There is, of course, a very specific circumstance surrounding Professor Taylor's unease. For the past two years he has been attempting to come to terms with a variety of phenomena that are usually put into the paranormal category. His motive has, he insists, consistently been to explain these phenomena by means of conventional scientific concepts. History is on his side in this respect; many phenomena which in the past were regarded as bizarre or supernatural have yielded to scientific analysis. History, as his critics continue to urge (see Professor Hammerton's letter on page 640), is not on his side in offering too much encouragement that professional scientists can outsmart professional deceivers, if deceivers some be.

The issue at hand, however, goes far beyond metal bending. Scientists are in an unusually favoured position in policing their profession, and so they have a special responsibility to keep these policing mechanisms up-to-date, fair and seen-to-be-fair. Governments devote vast sums of money annually to the practice of science, and even when the science is obviously directed at solving pressing problems, the scientist is still the ultimate arbiter of what he does and doesn't do. Indeed through a variety of mechanisms from refereeing for journals to sitting on grant-giving bodies, scientists are arbiters of what others are allowed to publicise and spend money on. Do scientists warrant the trust that the public, unwittingly, puts in them to keep their house in order and forward-looking?

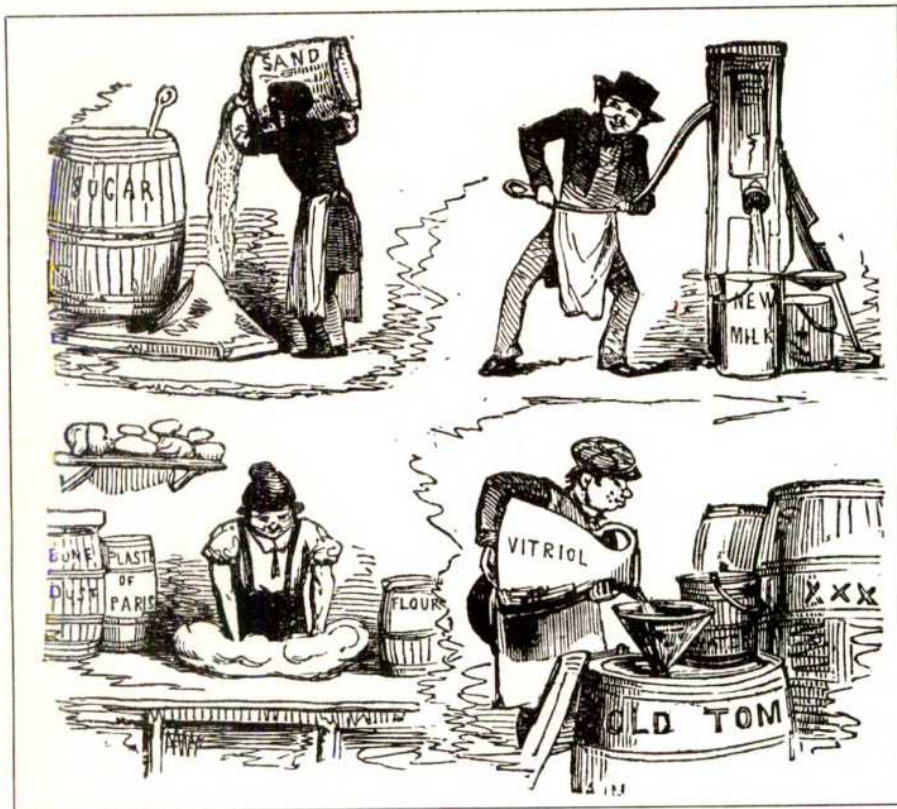
The greatest guarantee that the uncommon or unusual idea will get some sort of hearing is pluralism. If the sources of money are not controlled by just one committee, if the means of publication are not all in the hands of one person, if education is not according to some unified syllabus, then the prospect of any one group of people being able to exert an unhealthy influence is vastly diminished. And those who cannot get satisfaction through normal channels can often be remarkably effective in unusual ways. Research does

not have to have been sponsored by the Science Research Council to be distinguished, nor does it have to be published in *Nature* to command wide attention. Indeed some of those who feel they are unjustly ignored might be surprised how well known and extensively thought about their work is; simply because no-one relishes the thought of being known to posterity as the person who rejected a seminal idea. Likewise, as Professor Ball's letter (page 640) shows, experimental help does not necessarily come from a grant-giving body.

No doubt all of this sounds like smug self-satisfaction; the real test for the openness of the system, it will be said, is bound to be in the case histories rather than in assertions of fairness. The problem with case histories, however, is that conclusions have to be drawn from limited evidence and that there is often considerable difficulty in understanding the spirit of the time. Wegener's advocacy of continental drift in the early years of this century is often quoted as an example of an immensely important idea on which the establishment turned its back, thereby retarding the earth sciences by fifty years. But Wegener's ideas were well known and widely discussed; continental drift was the subject of at least one conference in the 1920s. Causes of drift were discussed extensively in the literature. What was missing was the compelling evidence that Wegener could not provide and that only an expenditure of large sums of money on marine research in the 1960s could. That example should warn us that certain fields can fail to flourish, even when confronted with an apparently blinding insight.

It should also warn that the case history is not an easy way of demonstrating inbuilt bias amongst scientists unless it is possible fully to appreciate the environment of facts, observations and speculations within which they lived at that time.

Science is ultimately about ideas moving around inside people. It does not reside in textbooks or in large pieces of equipment. Although it is possible for individuals to deny to other individuals the access to hardware or the literature through one particular channel, it is possible for no-one to control all channels, and it is certainly impossible to suppress ideas and people. This may be less true in other societies or in other professions, but surely the Western scientific community has had a relatively good record, and it is time that we stood up and said so. □



All the food that's fit to eat

During the latter half of the nineteenth century the public outcry about the adulteration of food in Britain led to the passing in 1875 of the first effective Sale of Food and Drugs Act. This Act, which is the basis of our present law, contained for the first time the requirement that all food sold must be of the nature, substance or quality demanded by the purchaser. It also required local authorities to appoint public analysts to check for purity samples of food, drink and drugs procured by local authority inspectors. To celebrate the centenary of the Act a symposium was held in London this week.

DURING this year's Annual Meeting of the British Association, Professor P. J. Newbould, of the University of Bradford, read a paper on the basic purpose to which the endeavours of modern science-based societies were directed. During mediaeval times, he pointed out, while nations struggled, as we do today, to be prosperous, the main aim of their citizens was salvation. In our own day it is economic growth and technological advance.

The symposium organised by the Ministry of Agriculture, Fisheries and Food to celebrate the centenary of the passing of The Sale of Food and Drugs Act 1875 was hardly intended to question that the continuous accretion of legislation and the elaboration of food standards and regulations, one following the other as analytical refinements and the minutiae of nutritional understanding advanced, is anything but good, nor that further control and standardisation in the future will be better. Indeed, the title of the symposium was "Food quality and safety—

a century of progress". Yet it is to be hoped that the sheer weight and size of the programme left time for someone to consider whether Harry Lauder's advice in the old song, to "keep right on to the end of the road" is always applicable. Perhaps, if we look around from where Professor Newbould is standing we may find that we are already approaching the end of one road and could usefully consider going in another direction.

In 1875, adulteration of food was rampant. Those were the days when "little drops of water, little grains of sand (made) the milkman merry, and the grocer grand". Something needed to be done to stop the watering of milk, the addition of alum to flour and even so flagrant a malpractice as the use of "sugar of lead", as lead acetate was called, to sweeten beer. Progress in analytical chemistry made it possible to police the 1875 Act. It is worth noting that the Society of Public Analysts, now the Society for Analytical Chemistry, was also established

in 1875 with Theophilus Redwood as its first president. The main purport of the Act was to decree that food on sale should be of "the nature, substance and quality" demanded. Provided his product came up to this straightforward standard, the merchant was, like other citizens of the realm, a free man innocently selling anything he pleased until he was proved guilty of marketing a product not of the "nature, substance and quality" his customer could reasonably expect. The march of time, the progress of science and the development of food technology in all its modern ramifications and, most important of all, a change in the philosophical outlook of the community, have led today to a radical difference in the approach of those who guide our society to what constitutes good wholesome food and the means by which these qualities can best be maintained. To start with, the analyst's ability to detect smaller and smaller traces of contaminants has enormously increased. Figures in terms of p.p.b. (parts per 10⁹) are as commonplace nowadays as p.p.m. once were and for some compounds analysts can measure as little as 1 part in 10¹². Then again, the much simpler problem of determining whether a particular consumer had or had not suffered from poisoning by lead acetate immediately after having drunk adulterated beer is now replaced by the exceedingly difficult one of assessing whether, because a proportion of a colony of rats to which Butter Yellow had been administered developed cancer of the bladder, people consuming foods coloured with Butter Yellow would within a period of years extending up to 70 (it has only recently been decided that if the postulated harm is forecast as likely to occur later than this it can be ignored—after all, it does not much matter if you are poisoned after you are dead) be likely to suffer also. Nor could it be assumed that people who wished to buy a wrapped white sliced loaf, or mayonnaise from which an oily layer would not separate, or a jar of "coffee whitener", or a carton of soft margarine, or a can of luncheon meat knew any longer what "nature, substance and quality" it was that they were demanding—or so those responsible for the public welfare led the public to believe.

During the hundred years since 1875, the whole community comprising the ordinary citizen, the scientist, the diligent guardian of the public good and the food manufacturer have all been carried together along the stream of advancing technology. No longer do any of these consider that an ordinary person can be left to himself to recognise a sausage or a pot of jam. Step by step the law has taken it upon itself

to set up quantitative standards of composition, to lay down detailed specifications for what may appear on the label or in an advertisement. That is why it is forbidden any longer to sell a "digestive" biscuit, since the digestibility of all biscuits may be assumed, while Bristol Cream, deficient as it is in butter-fat content, retained its label by special dispensation. Only approved colours, preservatives, flavours and other substances added to improve the texture or retain the moisture—all so properly described as "additives"—can today be added. And behind all this stands the scientist who alone possesses the skill to determine whether the law has been obeyed and the rules kept.

Refinements of the present rules are coming as we watch. Already shopkeepers are being required on pain of prosecution to sell some foods before a predetermined date although so far it is not actionable to be caught eating stale bread. And the Food Standards Committee, recognising that beans are different from meat, are proposing that, should a manufacturer fabricate beans into the semblance and texture of meat, he should mix with them a due proportion of the amino acid methionine.

Clearly, it is important to insure that food is wholesome, uncontaminated by toxic substances and not misleadingly labelled. The question to be asked now, after a hundred years spent applying more and more scientific ingenuity, is whether the means have been justified by the ends that have been attained.

Have increasing health and happiness followed each addition of sophisticated control and regulation? Do labels bearing lists of "additives" in small print contribute to the contentment of those who eat baked beans, fish paste and tubs of margarine? The unification of standards of food composition and of the analytical procedure needed to verify their maintenance, first within the country as a whole, next throughout the European Economic Community and finally, as soon as can be done, across the whole world, is already in hand. A council of wise men such as that set up by James I to prepare an English version of the Bible is well advanced in its work to prepare the *Codex Alimentarius*, the world gospel of food composition.

Although it is right and proper for all these great men, distinguished in science and prominent in government and administration, nationally, internationally and world-wide, to review the progress that has been made, the end of a century of effort to apply science to the control of food quality and safety is a good time to stop, just as Professor Newbould has done, not

merely to see how nearly the target—of perfect quality and absolute safety—has been approached but to consider whether there is perhaps a different target to be shot at. Do people fuss and worry about the safety and wholesomeness of their food in spite of the devoted efforts of the Food Standards Committee, the Food Additives and Contaminants Committee and the Labelling of Food Regulations or because of them? Is the health of the community safeguarded more effectively by forbidding the use of cyclamates on the basis of subtle circumstantial evidence based on animal experimentation on which scientific opinions is, to say the least, divided or, let us say, by making cyclamates compulsory in order to minimise the consumption of sugar which is known to be harmful? There are other questions, likewise never asked, of which the biggest is: since, of all the contributing factors, lack of money is the most potent cause of poor diet, should we not measure the cost of the vitamin enrichment of bread and margarine, of analysis for the presence of required ingredients and the absence of proscribed ones, of the listing of components on labels, of the wrapping and packing of this and the discarding and disposal of that, to try to strike a balance between the benefits all these measures bring and the price that has to be paid for them.

These, however, are technical questions and, whatever shortcomings there still may be and whatever questions still remain unanswered, the distinguished people who set up the standards, do the analyses, measure the risks and add the vitamins are above all competent in their fields. The philosophy behind their century of forward march is, however, that of science and, as it sometimes seems, of a science that is assumed to be capable of ensuring that absolute safety and perfect purity—standards to which in the end all the world's food must conform. In the century ahead there is room for a new idea.

Obviously, our food must be fit to eat and we need to apply scientific understanding to keep it so. But, since there is no such thing as absolute safety, any more than there will ever be complete knowledge, there is room in the next century for those who invent the food laws to allow some of the people some of the time to eat at their own risk. Perhaps if the scientific advisers and governors let it thus be known that British scientists know that they do not know everything nor ever will and that they are not able to avert every "Act of God", the ordinary citizen would be less inclined to blame the government for every misfortune.

Magnus Pyke

One step from chemical automatons

by Akiyoshi Wada

By anyone's standards, the revelation of the molecular mechanism of life must be considered one of the highlights of the history of natural science. To make this revelation more meaningful and beneficial to mankind, those engaged in research on biological molecules must begin to think of possible practical applications of the molecular mechanism of life. For this purpose, a committee was formed in Japan about a year ago to examine and explore industrial applications of biological molecules with special emphasis on enzymes and other functional biopolymers. This is one of the projects under the auspices of the Council of Science and Technology in Japan for the promotion of life science. The committee is still carrying out its work, but wishes to present an interim report on the general image of what these applications might be, in the hope that it will stimulate a response from those who are engaged in biopolymer research.

A LIVING body is a molecular machine whose parts are molecules; machines using molecules as functional parts are obviously far more compact than present man-made machines. We already know the functions of individual parts, which make up complicated yet ordered chemical circuits. We also know how higher order structures are constructed and regulated, how energy is converted from one form to another, and how information is transmitted in these chemical circuits. The knowledge of these functions and mechanisms serves as the basis for the next stage of our project: the development of a chemical automaton with highly developed self-contained chemical circuits.

Rather like an electronic computer, this automaton would convert input into output; in the automaton's case, however, input and output would be molecules. An electronic computer is made up of hardware and software; a chemical automaton needs an additional component, a chemical reaction

system which might be called 'wetware'. The functional molecular units of a chemical automaton serve for:

- Reaction.
- Regulation.
- Information storage.
- Material transfer.
- Separation of materials.
- Transport of energy.
- Conversion of energy.
- Detection of parameters at each of the reaction stages.

The ultimate automaton is likely to be what might be called a 'synthetic living body' which consists mainly of wetware and software. But, for now we should concentrate on developing automatons which also include hardware such as measuring devices, computers and energy sources. For this purpose we must first develop the following topics:

- Stable and specific catalysis.
- Specific selective membranes.
- Reaction control systems including measuring devices.
- Chemical circuit theories.
- Energy transducers.

Topics in basic research which need to be studied and therefore to be supported for the development of the above items are:

- Biopolymers, in particular the structure and functions of enzymes.

- Molecular mechanisms of specific reactivity and its regulation.
- Organic reactions in aqueous and neutral conditions (without strong acids or bases).
- Synthesis of polymers of determined sequence.
- Stabilisation and immobilisation of natural enzymes for easy use.
- Specific permeable membranes.
- Mechanism of energy conversion in biological systems.
- Stability of chemical reactions in relation to spatial and temporal chemical oscillations.
- Development of highly sophisticated measurement techniques in physics and chemistry needed for the aforementioned research topics. (For example, we can cite the most up-to-date electronic and mechanical devices and computers.)

Many obstacles lie in the way of the realisation of chemical automatons. They may seem insurmountable, but in comparison with dreaming of flying 100 years ago, or speculating on high speed computers 50 years ago, chemical automatons are only a step away. In any event, nature provides chemical automatons as living bodies, and the principle behind their working mechanism is fairly well understood. Thus, the obstacles are indeed surmountable,

and efforts made in overcoming these obstacles are well worth exerting, when we think of the beneficial effects of chemical automatons on society.

Some of the direct effects would come from a new form of chemical industry, operating at normal temperature and pressure in the state of aqueous solution, requiring minimal energy and producing no harmful by-products. Other benefits would be flow production of complex chemicals which does not disturb the environment, and the possibility of the full use of substances abundantly available in nature, such as air, water and carbon dioxide.

Indirectly, chemical automatons would affect both basic and applied sciences covering a wide range of fields such as biophysics, molecular biology, biochemistry, polymer chemistry, the textile industry and the pharmaceutical industry.

In the advanced nations, including Japan, the potential for the development of chemical automatons is great. Barriers do, however, exist for such people to switch their specialities to life science. The automaton project straddles over many academic disciplines, and it is hoped that it could serve to trigger meaningful interdisciplinary research centring on the molecular life sciences. □

international news



Eight share Nobel Prizes

DURING the past week, Nobel Prizes for medicine, chemistry and physics, at £69,000 tax-free a time, have been shared by eight European and US scientists. The prize for medicine was shared by Renato Dulbecco, a 61-year-old Italian-born American now working at the Imperial Cancer Research Fund, London, and two of his former pupils—David Baltimore, aged 37, of the Massachusetts Institute of Technology, and Howard Temin (40), of the University of Wisconsin.

Baltimore was Dulbecco's student at the Salk Institute for Biological Studies, and Temin studied under him at the California Institute of Technology. They receive their prize for their work on the circumstances and effects which cause cancer. Dr Dulbecco's work has been on the mechanics by which animal viruses insert their own genetic material (DNA) into that of

animal cells. This can enable them to convert the cell into a virus factory, but it can also transform the cell into a cancerous state.

Dr Temin and Dr Baltimore share the credit for an outstanding discovery about RNA tumour viruses. RNA usually acts as the messenger molecule by which the genetic instructions of body cells are translated into proteins. Dr Temin and Dr Baltimore were convinced that RNA tumour viruses must be able to reproduce themselves in a DNA form to enter the cell's genetic machinery. Ten years after Dr Temin first predicted it, both men almost simultaneously found the enzyme that enables them to do it. The enzyme enables the virus to make DNA from RNA.

The discovery stood conventional biology on its head because it reversed the genetic process by which DNA makes RNA and RNA then makes protein.

Many RNA viruses are involved in animal cancers and it is believed that some human cancers may be caused in the same way. Work with these viruses

has also shed considerable light on the basis of the genetic workings of cells.

The chemistry prize was shared by John Cornforth (58), now at the University of Sussex, and Vladimir Prelog (69), a Yugoslav-born Swiss citizen who teaches organic chemistry at the Federal Institute of Technology in Zurich. The Royal Swedish Academy of Sciences said that Cornforth, who has been deaf since childhood, had made "an outstanding intellectual achievement" in his work on the stereochemistry of enzyme-catalysed reactions.

Professor Cornforth has used enzymes, which are natural biological catalysts for speeding chemical reactions, to look at the synthesis of biological molecules. In particular, he has shown the steps by which cholesterol is made.

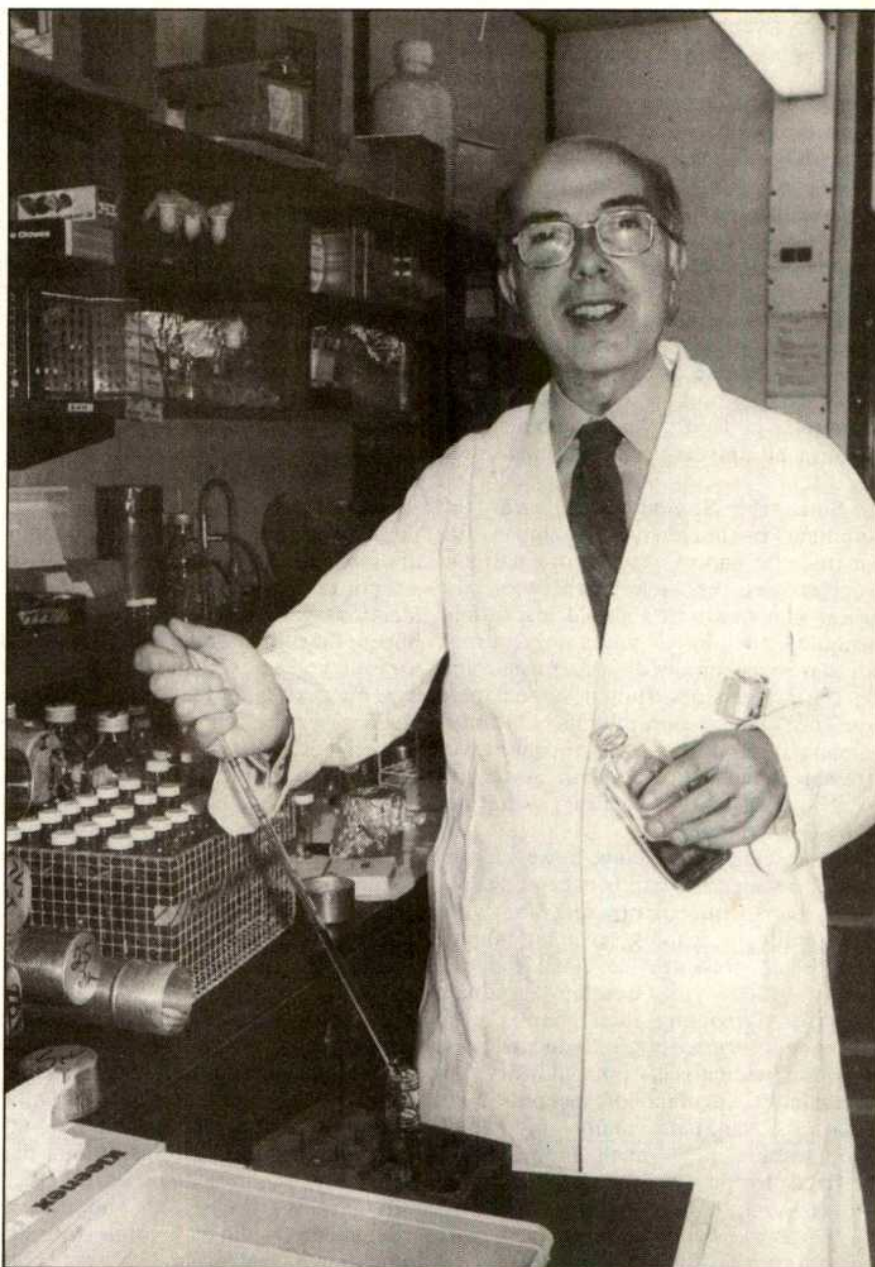
Dr Prelog has contributed to an understanding of how natural molecules are often produced in an asymmetrical fashion. Many molecules can exist either in a "right-handed" or in a "left-handed" form. Artificial manufacture of such molecules generally produces equal amounts of each. But in nature, only one is produced.

An American and two Danish nuclear scientists shared the physics prize for research into the structure of atoms. They are James Rainwater, of Columbia University, New York; Aage Bohr, of the Niels Bohr Institute, Copenhagen; and Ben Mottelson, of Nordita, Copenhagen.

Rainwater, Bohr and Mottelson won their prize for the invention of the 'collective' model of the nucleus. This is a synthesis of two earlier models: the liquid-drop model (a rather coarse, very 'collective' model in which the nucleus is pictured as a charged drop which deforms as a whole; it was very successful in explaining the masses of nuclei and fission) and the shell model (in which nucleons are pictured as existing in individual orbits and forming closed shells; this leads to the idea of 'magic numbers'—particular numbers of nucleons conferring exceptional stability on the nucleus).

The latter model was much more detailed in its treatment of the nucleus, and one could derive much more information about excited states from it. Experimental deficiencies were observed, however; many nuclei exhibited much greater quadrupole resonances than could be explained on the shell model, and also some nuclei showed an excited level structure reminiscent of rotational bands. The collective model pictures the outer nucleons, in individual orbits, interacting with the closed inner shells and deforming them—this gives the 'collective' effect, and achieves the synthesis of the two models. □

Dulbecco, pictured at ICRF this week



Howard Temin



David Baltimore

Education issues in Japan

ONE of the most commonly quoted statistics about Japanese higher education is that graduates comprise only about 3% of total student numbers (compared, say, with the UK where graduates comprise 20% of all students). What is less often mentioned is that even this 3% meant 46,000 graduate students in 1973—more than in the UK. Even so, the concept of the graduate course as an integral part of the structure of a university is less common in Japan. As the table shows, half of all universities do not have such courses.

Since the Second World War the number of universities in Japan has increased enormously. For national universities the government took the view that newly established institutions should not have graduate courses; rather, the courses in the former imperial universities should be enlarged in size and scope. Some additional financial support was forthcoming for research and education, but in general staff numbers and facilities were not allowed to increase.

This selective funding, however, was seen to widen the gap between research activities in universities with and without graduate schools, so great, almost fanatical, pressures developed in the early 1960s to develop graduate courses. Originally such courses were intended primarily to educate new research scientists, but industry increasingly demanded people with master's degrees, many of which courses are now much more closely related to production processes than to research.

The market for people with doctorates is limited to universities and the research institutes of government and private industry. But enrolment in higher education seems to be reaching saturation, so universities are no longer taking on many new staff; the same is true of most other institutions, with exceptions only in the environmental and social sciences. The problem of the so-called 'over-doctors' is serious, and many have proposed an increase in the number of postdoctoral fellowships as a way of preserving a reservoir of highly qualified manpower for future demands.

The Japanese concept of the chair (*koza*) undoubtedly had its origins in the European professorial chair, although there are many differences.

It is the structural unit of the faculty, responsible for education and research in a specific subject area, and is composed basically of one professor, one assistant professor and two assistants. It is provided with its own running expenses and facilities. Although often criticised as an indestructible castle of conservatism and hierarchy, it functioned reasonably well in the pre-war imperial university, and has also been fitted into the present frame-work of graduate education. Partly because of the rapid expansion of the size of enrolment, and partly because of the enormous development of the sciences, the idea emerged that it would be more desirable to lift the restriction that the structural unit for education should be identical with that for research. It was hoped that this would make teaching as well as research more flexible and dynamic. After lengthy discussions, the laws governing the structure of the universities, both national and private, have been modified to allow the possibility of avoiding the traditional structure of the faculty based on the *koza* system, and to admit a more flexible one. Any new venture has to be discussed with the Ministry of Education, of course.

One of the issues from a recent report from the University Council (*Daigaku-setchi Shingikai*) in the Ministry of Education is the possibility of independent graduate institutions outside the universities. Although apparently a reasonable proposal, and even though such an institution may be highly qualified in research, there still needs to be an appropriate mechanism to link research with the educational background characteristic of a university. The lack of a mechanism for encouraging interaction between research and education could be harmful. The report also mentioned that it would be worth considering associated graduate institutions administered through the cooperation of several universities. Serious efforts are being made in certain groups of universities (including cooperation between national and private universities) to encourage the exchange of teaching staffs or of student credits. These changes will be particularly useful in developing interdisciplinary courses such as information science or environmental science.

from Yoshinoku Kakiuchi, Tokyo

	Total no. of universities	master's degrees only	Universities offering	
			master's degrees and doctorates	doctorates only
National	81	38	26	1
Public	33	4	8	7
Private	299	37	72	12
Total	413	79	106	20

Anti-nuclear critic faces dismissal

by Allan Piper

A LEADING West German nuclear scientist is faced with dismissal from his chair at the University of Bremen, apparently because of his support for the anti-nuclear energy lobby. Dr Jens Scheer, Professor of Nuclear Physics at Bremen and a state parliamentary candidate for the German communist party (KPD), has claimed that the action against him arises out of his active criticism of the West German nuclear programme, and that it is not a consequence of his political affiliations, as is officially claimed.

Professor Scheer was suspended on September 23 following his alleged involvement in a disturbance on the university campus during which the officials of a civil court were attacked by egg throwers. Though he denies the charge he will now face a special disciplinary tribunal. The University authorities have stated that they are seeking his dismissal, the official reason given being that Professor Scheer, as a member of the KPD, has contravened "the code of honour which every [civil servant] must, through his whole behaviour profess and seek to maintain". Although as head of a university department Professor Scheer holds what is effectively a life appointment under existing German law, his dismissal on those grounds would be perfectly in order. A growing body of support for him maintains, however, that a little used legal clause has been invoked to mask the real reason for dismissal.

Professor Scheer's supporters, including a large number of eminent European academics and political organisations, believe that in seeking to remove him from his position at the university, the authorities hope to overcome a potentially powerful source of opposition to the West German nuclear energy programme. As yet Germany has no nationally coordinated anti-nuclear lobby but in the past Professor Scheer has often provided expert support for small, local protest organisations. Further, since his arrival at Bremen in 1971 Professor Scheer has established his department as a centre for the study of the interactions between science, technology and society, and in May this year the department published a book examining the strategy and consequences of nuclear development.

● Yesterday it was learnt that Professor Scheer's suspension has been overruled by a court of law, allowing him to resume his normal duties at the university, but dismissal proceedings will still go ahead. □

AN international group of scientists involved in bacteriophage work is unhappy about the way in which the Recombinant DNA Molecule Program Advisory Committee is handling the problems of plasmid engineering, and a meeting of the committee planned for this month has been put off until December because of the concern which has been expressed about its last meeting at Woods Hole.

The group, which consists of 48 scientists who attended the recent Cold Spring Harbor bacteriophage meeting, has sent a petition to the National Institutes of Health (NIH), complaining that the Woods Hole guidelines on recombinant DNA represent a watering down of the recommendations made at the Asilomar conference earlier this year. The Asilomar conference, convened by Professor Paul Berg, was the first gathering of workers in the field to discuss the potential dangers of plasmid engineering, and a subsequent meeting of UK workers at Oxford broadly confirmed the working rules suggested at Asilomar.

In a letter to Dr Dewitt Stetten, Deputy Director for Science, Office of the Director, NIH, the Cold Spring Harbor people complain that a draft of the Woods Hole meeting called "Current Guidelines for Research on

Recombinant DNA Molecules" appears "to lower substantially the safety standards set and accepted by the scientific community as represented at the meeting at Asilomar in February, 1975".

DNA committee has its critics

The letter "strongly requests" that the advisory committee considers at its postponed meeting the feelings of the group in three areas.

- They urge that the most hazardous experiments be curtailed until some experimental determination of the risks inherent in such procedures is made. They say, for instance, that the extent of containment possible with different vectors remains to be shown.

- They are concerned that any mammalian DNA (let alone animal viral DNA) can, by the present draft, be cloned under less than P3 containment, and they say that they are not persuaded that an untested vector designed for safety reasons is by itself an adequate safeguard for such experiments in an open laboratory. They add that "strong consideration should be given to limiting shotgun experiments of mammalian DNA to P4 contain-

ment until proven safety vectors are available".

- They feel that the composition of the committee should be broadened to include more representation from the areas of animal virology, plant pathology and genetics, and epidemiology; and also that the advisory committee should have much stronger representation from scientists not directly involved in cloning experiments. And taking a line which the committee will surely find hard to swallow, they think it advisable "to consider representation of the public at large".

One of the organisers of the petition, Richard N. Goldstein, of the Harvard Medical School, says the letter "reflects a deep concern" with the results of the Woods Hole meeting, and he believes it is "exceedingly important that the general scientific community be made aware of these developments".

- This week Dr Goldstein reported that the Woods Hole guidelines had been scrapped. According to Goldstein, Dr Betty Kutter, "a vocal critic of these guidelines and a member of the Recombinant DNA Molecule Committee, has been charged with the re-writing of these guidelines as a result of the pressure put on the committee by many dissatisfied scientists".

EVER since the days when medicine was largely the province of hucksters and snake oil merchants, people have been touting cures for cancer. Nowadays, such cures may be mentioned briefly in a racy tabloid newspaper, but they are usually ignored by self-respecting scientists, attract mercifully little following, and are quickly forgotten. But not so with one purported anti-cancer remedy called Laetrile.

Although declared contraband by the federal government, outlawed by several state governments and found to be utterly worthless in a number of tests carried out at several prestigious cancer research institutes, Laetrile is now being consumed by an estimated 20,000 people in the United States. It is available on the black market, or through clinics in Mexico and West Germany, to which desperate American cancer sufferers are flocking in droves. It owes its popularity in the United States to a vocal, and at times heated, campaign by a number of groups on the West Coast who are fighting to get legal restrictions on Laetrile lifted.

The bitter battle over Laetrile would have all the ingredients of a good thriller, if the subject matter were not so tragic. Research reports have been stolen and given wide publicity, an international smuggling ring has been broken up by federal agents, cancer

Trials for Laetrile

by Colin Norman, Washington

researchers have been accused of deliberately suppressing information, numerous court fights have occurred, and right-wing political groups have been accusing the government of invading personal freedom. The matter has certainly caused a headache for the Food and Drug Administration and the National Cancer Institute, and a good deal of embarrassment for the Memorial Sloan-Kettering Cancer Center in New York.

Laetrile was apparently first used for cancer treatment in the 1920s by a California doctor called Ernest T. Krebs, Sr, but it was too toxic to be much use. A purified form was developed in 1951 by Krebs' son, E. T. Krebs, Jr, a biochemist, who claimed that the substance was safe for injection. More recently, Laetrile has been produced in a form which can be taken orally, and its use has skyrocketed.

There have been numerous anecdotal reports of cancer sufferers who have gone into remission after taking Laetrile, or who have at least experienced a cessation of pain and have died in relative peace. But there have been no formal, clinical trials to test the efficacy of the substance, and until recently

there have been few animal trials to test Laetrile's purported anti-cancer activity. Results of two extensive animal trials will, however, be published later this month. They are unambiguously and crushingly negative.

Proponents of Laetrile have even suggested an elaborate mechanism to explain its alleged action. The substance, they suggest, is broken down inside cancer cells by the enzyme β -glucosidase, to release benzaldehyde and hydrogen cyanide in sufficient quantities to kill the malignant cells. Normal cells, they suggest, are protected because they contain the enzyme rhodanese which, in the presence of thiosulphate, converts hydrogen cyanide to the less toxic thiocyanate. It is a neat mechanism which every cancer chemotherapist looks for—something which is entirely specific to cancer cells but non-toxic to normal cells. The trouble is, though, that there is not a shred of evidence so far to support it.

The campaign in support of Laetrile certainly has considerable popular appeal. A film developed for the pro-Laetrile forces, for example, begins with the following statement: "This year, 250,000 Americans will die from cancer . . . this great human tragedy can be stopped now entirely on the basis of existing scientific knowledge". It goes on to note that "the history of science is the history of struggle

against entrenched error", and that some of the greatest scientists were ridiculed in their own time. It states that "with billions of dollars spent each year on research, with other billions taken in from the cancer-related sale of drugs, and with vote-hungry politicians promising ever increasing government programs, we find that, today, there are more people making a living from cancer than are dying from it", and argues that scientists therefore don't really want to find a cure for cancer because "if the riddle were solved by a simple vitamin [Laetrile], this giant commercial and political industry could be wiped out overnight".

In 1972 and 1973, the campaign took a new tack. Petitions, said to be signed by 43,000 people, were sent to former President Nixon demanding that clinical trials be run on Laetrile to test its anti-cancer properties. The petitions were responsible for launching four animal studies: two were carried out under contract to the National Cancer Institute (NCI), and two others were performed at the Memorial Sloan-Kettering Cancer Center and the Catholic Medical Center in Queens, New York.

Results of the two NCI studies will be reported later this month in the September/October issue of *Cancer Chemotherapy Reports*, a journal published by the NCI. Conducted by Isidore Wodinsky and Joseph Swiniarski at Arthur D. Little Inc. (ADL) in Cambridge, Massachusetts, and by W. R. Laster, Jr, and F. M. Schabel, Jr, at the Southern Research Institute (SRI) in Birmingham, Alabama, the studies involved large doses of Laetrile administered to mice bearing transplanted tumours. A standard method of screening for anti-cancer drugs, it consists of transplanting pieces of tumour from one mouse into other, genetically identical, mice and observing the effects of drugs on tumour growth. The ADL studies involved four different types of cancer, and the SRI tests three.

According to Wodinsky and Swiniarski, Laetrile did not prolong the life span of mice bearing any of the transplanted tumours, even at doses close to the level at which the substance was found to be toxic. They therefore conclude that "no anti-tumour activity was found in any of the four systems tested". Similar results were reported on the basis of the SRI tests. Laster and Schabel conclude that Laetrile "did not demonstrate significant anti-tumour activity against any of these three tumour systems".

Such negative results would normally be sufficient to close the book on a possible anti-cancer agent, but not Laetrile. The studies at the Sloan-Kettering have, inadvertently, provided

some ammunition for the pro-Laetrile forces.

According to Dr C. Chester Stock, a vice-president of Sloan-Kettering, and director of the institute's Walker Laboratories, his institute took a brief look at Laetrile in the early 1950s, but found no effect when it was tested against one implanted tumour system. The work was then dropped because "other agents were showing promising activity", he said. But when the petitions started flooding into the White House and letters began arriving at the Sloan-Kettering, demanding that Laetrile be tested, it was decided that the institute should take a second look at the substance. Particularly pertinent to that decision was the fact that the chairman of the President's Cancer Panel, Benno C. Schmidt, is also a board member of the Sloan-Kettering Institute (SKI). According to Stock, tests were run on "a battery of transplanted tumours" at high doses, in 1972, but he said "we didn't see any benefit whatsoever"—Laetrile again proved to be ineffective as an anti-tumour agent.

Although the use of transplanted tumours is a standard procedure for testing anti-cancer drugs, it has recently come under considerable criticism on the grounds that transplanted tumours may not behave like spontaneously occurring ones. The decision was therefore made at the SKI to test Laetrile on a strain of mice (developed by Dr Daniel Martin at the Catholic Medical Centre) which has a very high incidence of spontaneous mammary tumours. The tests were begun in 1972 by one of the SKI's most venerable researchers, Kanematsu Sugiura.

Sugiura's initial studies seemed to show a surprising effect. Although Laetrile apparently had only a small and variable effect on the primary tumours, it seemed to have a marked effect on the appearance of lung metastases. According to tests run between September 1972 and June 1973, Sugiura found that whereas 78% of the mice in control groups had lung metastases present, only 17% of those treated with Laetrile developed metastases. Sugiura wrote up the results of his experiments in a report which he circulated to his colleagues.

In the autumn of 1973, somebody at the SKI sent a copy of Sugiura's report to Laetrile proponents on the West Coast, and it subsequently got banner headline treatment. Supporters of Laetrile began to claim that the prestigious Sloan-Kettering Institute had proven the effectiveness of Laetrile, and that the institute was suppressing the results.

Since that time, Sugiura has continued to study the effect of Laetrile

on spontaneous mammary tumours in the mice bred by Martin, and parallel tests have been run by two other researchers at the SKI and by Martin himself. Sugiura has continued to find that Laetrile seems to inhibit the formation of lung metastases, but the other three investigators have all come up with negative results. Reports of Sugiura's recent research have been circulated within the SKI, and a month or two ago they were again leaked to Laetrile proponents on the West Coast, who distributed them widely to the general press.

In view of the wide publicity which the studies have received, and the charges that the SKI has been suppressing results favourable to Laetrile, Dr Stock recently discussed the issue.

Stock said that "because of the emotion surrounding this issue, we felt that we should be cautious". It was agreed that Sugiura and Martin should cooperate in a test on Laetrile in mid-1974. The experiment proceeded in exactly the same manner as Sugiura's earlier studies, except that lungs from the mice were subjected both to microscopic examination and to bioassay—the lungs were transplanted into a fresh animal to see whether tumours grew. Bioassay provides an objective assessment of whether or not lung metastases were present. The results proved negative—there was no difference between mice treated with Laetrile and the controls. Similarly, Stock said that the two other SKI studies proved to be negative.

Thomas emphasised that the SKI has certainly made no attempt to suppress results favourable to Laetrile, and he said that "I think it is safe to say that if it had been any other agent under study, we would have lost interest in it" when Sugiura's early results seemed to indicate that it had little or no effect on primary tumours.

The fact remains, however, that in spite of a large, and growing, body of information which indicates that Laetrile has no anti-tumour effect in animal systems, the substance is continuing to grow in popularity, and its proponents remain convinced of its efficacy. They are still demanding that human trials should take place.

Most cancer specialists are adamant that clinical trials are certainly not justified. Thomas, for example, said that "in the absence of positive data from animal experiments, use of Laetrile in human beings with cancer remains extremely difficult to justify, particularly in patients with cancers that are treatable by surgery, radiotherapy or chemotherapy". The Food and Drug Administration (FDA), which would have to licence any clinical trials, agrees. An FDA official pointed out that it would be difficult to

It is not, I hope, a serious contravention of the Official Secrets Act to reveal that, in Committee Room 11 in the Houses of Parliament, the wall above the rostrum is adorned with an enormous oil painting which depicts "The Speakers Procession in 1884". The Parliamentary and Scientific Committee sometimes meets in this room, and when the proceedings are less than enthralling I find myself studying the picture. My interest is not so much in attempting to identify the impressive figures (the print on the key diagram is too small to be read at a distance) as in wondering at the appalling mess of litter and waste paper through which the procession is wading. It is almost like the platform of a London underground railway station in 1975. I am glad to say that the corridors of power are, in this respect, more hygienic today, if other places are becoming filthier.

Litter and refuse are undoubtedly providing some of our most difficult pollution problems today. Both New York and London seem to be permanently subject to piles of stinking refuse, even when there is no industrial dispute between the local authorities and the refuse collectors. Earlier this year the strike of dustcart operators in Glasgow faced the city with a serious public health problem, so that the army had to be called in to clear away the more noisome dumps. The problem is made worse by the way in which the bulk of the litter is swelled by masses of unnecessary packing material, but its danger is also a function of affluence, for it is the waste food, much of it initially quite fit for consumption, that makes refuse a public health problem, attracting rats and increasing the risk of transmission of disease.

What may not be generally realised is that it is the successful control of air pollution which exacerbates the problem. Earlier this century the volume of refuse was much smaller, partly because much was burned on domestic grates and kitchen ranges.

It is true that some of the more

affluent do have refuse disposal units, to pulverise food and some other wastes, which are then discharged into the sewers. This may allow their hygienic disposal, though there have been reports from America of a consequent overload of sewage works and even more serious pollution of rivers when the material is discharged, untreated, into them. We are often better at moving pollution from one place to another than in curing or controlling it.

The Chief Scientist to the Ministry

Waste line



KENNETH MELLANBY

of Agriculture, Fisheries and Food, has recently stated that, of the food bought in Britain, as much as a quarter may be wasted. This does not include the substantial amount that is eaten, over and above their actual requirements, by most of our population, contributing to the obesity which is the most serious symptom of malnutrition in most Western countries today. It seems likely that, if we eliminated waste and gluttony, we could reduce our balance of payments deficit by nearly £1,000 million, without any marked change in our feeding habits.

Although there is a growing interest in recycling all types of waste material, little food is re-used in this way. In the past, much pig swill came from hotels, schools and even from private houses. Since swine vesicular disease has

become a serious problem, such collections of swill have greatly decreased, as regulations to ensure that the food is properly treated and sterilised, though wise and necessary, are too stringent for the majority of small operators. It is calculated that the dustbins of Britain contain at least 3 million tons of "putrescible material" (the trade jargon for waste food) a year, much with a high protein content, and that if this were properly treated it could feed more than 1 million pigs—as well as reducing the health risks arising from uncollected refuse.

If waste food is to be used to feed animals, it has to be separated and treated. There are less difficulties in recycling refuse as a soil fertiliser or conditioner. Considerable amounts of sewage sludge are used in this way, and several cities have installed elaborate and expensive plant to produce municipal compost. Wastes brought in by the dustcarts are sorted and separated (tins and iron scrap is removed magnetically, baled and sold) and the remainder, with added sewage sludge, goes through a digestive process. The end product is a brown powder which is quite pleasant to handle. It contains a significant amount of nutritive salts, and is an excellent conditioner for intractable clay soils. Although available at a low cost, most farmers have been reluctant to use municipal compost, because it usually contains substantial amounts of heavy metals, the lead level commonly exceeding 200 p.p.m. There is evidence that, in the presence of a high level of organic matter, this lead is hardly taken up by most plants, but it does remain in the soil and might give rise to problems at a later date if the organic level fell substantially.

Waste disposal and re-use is thus seen to be a very complicated problem. Clearly the best solution is to produce less, and to see that what cannot be avoided does not give rise to further problems. But it is at least encouraging that our present Members of Parliament are better house trained than their Victorian predecessors.

design an acceptable clinical trial without depriving patients of other, accepted methods of treatment. In other words, if Laetrile were put on clinical trial, it could only be justified ethically if it were simply added to other forms of treatment, and that wouldn't make for a very rigorous test.

There is, however, another extremely important aspect to the Laetrile debate. Laetrile, in its purified form, is relatively non-toxic. It can be fed to animals in large doses before any adverse effects are noted; according to Thomas, at least one of the "solid

pieces of data" to come out of the SKI studies is confirmation of that fact. Laetrile proponents therefore argue that FDA should not regulate the substance as a drug, but as a food or even a vitamin.

Would it do any harm if the FDA were to allow Laetrile to be marketed like any other so-called health food? Even the *New York Times* has suggested, in an editorial published in August, that since Laetrile is usually taken by cancer patients who are beyond help from conventional therapies, they should not be denied even a

possible placebo effect from the substance. But a letter from Dr Sherwin Gardner, Deputy Commissioner of the FDA, took exception to that suggestion. Pointing out that the FDA has a duty to protect the public against ineffective drugs, Gardner said he believes that "the idea of fraudulent promotion and sale of bogus cures to the desperately ill and dying is appalling". It is also argued that, if Laetrile is made more widely available, it will be taken by cancer patients in preference to other therapies which have at least proved effective in the past. □

correspondence

Laboratory charges

SIR,—I am much in sympathy with Kenneth Mellanby (October 2) and especially his final paragraph on inexpensive biological research. Having simple research needs—eyes, a waterproof and time for fieldwork and contemplation—I have been conscious throughout my career as a littoral ecologist, of the patronising designation 'only a naturalist' from those who think that little of value can be achieved without, for example, an autoanalyser, an atomic absorption spectrophotometer or an electron microscope. Tempted though I have been at times to try to impress visitors with a benchful of borrowed equipment and some coloured liquids their attitude has nevertheless remained as water on a limpet's back, and the major events of recent years have finally reinforced my conviction that a field of biology that is important but relatively inexpensive, that appears simple but is very challenging, has been ignored in favour of the esoteric and the elaborate.

This event is the 'ecological crisis', and more particularly within my own discipline the dawning realisation that by generally eschewing organismal biology for several decades marine biologists are not now able to tell society with conviction the state of affairs in the sea or along the coast. Time after time one is asked if pollution is affecting local populations, if a scarcity of this or an abundance of that is natural or not. With embarrassment one admits to ignorance, except in obviously severe instances, and then, in response to mutterings about taxpayers' money and what biologists have been doing all these years, one loyally defends colleagues who did not find it worthwhile intellectually or in career terms to count organisms, or to study their biological interactions, their reproductive cycles, their behaviour or even how long they live—all of which would have better enabled us to assess the present field situation and respond to the public concern. But attitudes are changing; the doom-mongers have, after all, done biology a service.

To those who may wonder what to turn to if their capital investment should be rendered valueless by an inability to meet the unforeseen but swingeing maintenance contracts, may I offer a suggestion? Lend a hand on a field course and encourage accurate

observation and speculation about what organisms do, how they live in a complex natural situation. The range of as yet unanswerable questions about even the most common species that will surely arise from a class of enquiring minds will not only be humbling but will point to many rewarding research projects. In short, natural history appears in many up-dated guises but still presents unlimited and relevant challenges.

Yours faithfully,

J. R. LEWIS

*Wellcome Marine Laboratory,
University of Leeds, UK*

This week's free offer

SIR,—In a recent BBC "Controversy" programme, Professor John Taylor expressed strongly his opinion that scientists were stifling science. One of his points of contention was that he had been unable to obtain a grant from the Science Research Council to support investigations into the supposed paranormal phenomena associated with Uri Geller and others, including some children. In discussion he said that he had not the facilities for a thoroughgoing scientifically controlled experiment and mentioned that these could include such expensive items as electron microscopes and audiovisual recording equipment.

The phenomenon most frequently mentioned is the deformation and fracture of metallic objects without the application of the relatively large stresses normally to be associated with such changes in the metal shape. If it were true and could be substantiated, this would be an entirely new metallurgical phenomenon.

The equipment required for its investigation, including all the items mentioned by Professor Taylor, is available in this department and could be used for a definitive experiment. I would therefore like to invite Professor Taylor to join with us in devising a controlled experiment which would test the authenticity of the claims made by those, and particularly Uri Geller, who say that they can bend metallic objects without the direct application of the stresses normally required.

I would suggest that to establish the experiment's authenticity, its control and observation by independent witnesses should be approved beforehand by yourself and/or a nominated panel

of scientists and conjurers. The experiment could be devised to be definitive and its cost would be very small in relation to the importance of the claims that have been made. Professor Taylor need have no recourse to financial resources as the conduct of the experiment would be within the normal research activities of a department such as this. I very much hope that Professor Taylor will be willing to take up this proposal.

Yours faithfully,

J. G. BALL

*Department of Metallurgy and
Materials Science,
Imperial College, London, UK*

Cheating children

SIR,—Professor Taylor has cited a remark of Sir William Crookes on the relative susceptibility to deception of physicists and conjurers. It would materially help in weighing this statement if he would tell us whether it was uttered before or after Sir William had disgraced the scientific calling by becoming an accomplice of the fake medium Eliza Cook; if the latter, was it before or after the conjurer Maskelyne indicated the mechanism of deceit?

Yours faithfully,

M HAMMERTON

*University of Newcastle upon Tyne,
UK*

Out of town

SIR,—May a humble linguist point out that "commanded out" ("Publishing problems", September 4) is probably an attempted translation of the German *abkommandiert*, which is a military (and probably bureaucratic) term meaning "temporarily transferred" to another place ("detached" is the English term as opposed to a permanent "posting"). Central Europeans, particularly scientists and technologists, often use German as a lingua franca; and central Europeans tend to be rather 'disciplined' in their jargon as opposed to the trendy Anglo-American 'free-and-easy' style. It is all a matter of custom. Without prejudice to your correspondent's point, his friend may merely have meant to say "I was out of town for a couple of weeks, and couldn't reply earlier".

Yours faithfully,

A. LODGE

Swanley, Kent, UK

news and views

Recent developments in scrapie

from Richard H. Kimberlin

FOR over a decade scrapie has excited the interest of scientists in all parts of the world because of the unusual nature of the disease and its causal agent; few diseases have aroused so much discussion and speculation.

Originally scrapie was studied as an important disease of sheep which, after a very long incubation period, can be recognised by the characteristic signs of uncoordinated movement and "scraping" or rubbing of the skin. These signs are associated with damage to the brain and, under the light microscope, the brain lesions appear mainly as vacuolation. It is now recognised, however, that sheep scrapie is the best known member of a larger group of diseases that includes transmissible mink encephalopathy (TME) and the two diseases of man, kuru and Creutzfeldt-Jakob disease, all of which have a similar brain pathology. Some recent studies of scrapie and TME in golden hamsters have shown that these diseases are almost indistinguishable either histologically, biochemically or biologically when compared in a common experimental host (Kimberlin and Marsh; Marsh and Kimberlin, *J. infect. Dis.*, **131**, 97-103, 104-110; 1975). It now looks as though scrapie may be important in studies of an even wider group of diseases because some strains of the scrapie agent can produce cerebral amyloid deposits in mice which are broadly similar to the amyloid plaques seen in aged human beings and in some neurological disorders of man such as Alzheimer's disease and Down's syndrome (Bruce and Fraser, *Neuropathol. appl. Neurobiol.*, **1**, 189-202; 1975).

Most of our knowledge of scrapie has come from studies of the disease in mice. The length of incubation is influenced by many factors such as the amount of agent and the genotype of mouse and, depending on these variables, it can be as short as 100 days or may exceed the lifespan of the host (Dickinson *et al.*, *Nature*, **256**, 732-733; 1975). Particularly impressive is the remarkable predictability of incubation period under defined conditions of infection. In most cases the incubation time can be measured with a standard error of only 1 or 2% of the mean suggesting an almost clockwork-like precision in the development of disease. Multiplication of the agent occupies much of the incubation period. When peripheral routes of infection are used the agent multiplies initially in extra-

neural tissues such as the spleen. Only later does agent become detectable in brain and the amount of agent increases slowly until clinically recognisable disease develops.

There is little doubt that the long incubation time is due to the slow rate of agent multiplication in brain. It is known that the dynamics of agent multiplication are under the control of a gene called *src* and there is increasing evidence that the slowness of the process is due to a host restriction on the number of replication sites. The most convincing evidence of this comes from the demonstration that the previous injection of a strain of agent that is "slow" in a given mouse genotype can completely block a "fast" agent injected later (Dickinson *et al.*, *Nature*, **253**, 556, 1975). The interference phenomenon is most simply explained in terms of the "slow" agent occupying replication sites which would otherwise be available to the "fast" agent.

To appreciate the significance of these findings it should be emphasised that no humoral or cell-mediated response to scrapie infection is apparent during the incubation period, that is, there is no evidence that "competition" between agents has an immunological basis. Similarly, prolonged depression of T or B lymphocyte functions has no effect on incubation period and there is substantial evidence that interferon plays no significant role in scrapie pathogenesis.

Despite the limited role of conventional host defence mechanisms there are clear indications of some kind of scrapie inactivation system in mice. Moreover, the importance of spleen (and probably other extraneural tissues) in the early multiplication of agent indicates the importance of some components of the lymphoreticular system. The question is—which cells are involved in these scrapie-inactivation and scrapie-multiplication processes?

Even though there is no evidence of an inflammatory reaction in scrapie brain there is a suggestion that an in-

flammatory response may be important because it is now known that injection of mice with prednisone acetate or with arachis oil (both of which suppress inflammation) can prolong the incubation period (Outram *et al.*, *Lancet*, **i**, 198-200; 1975). It is particularly interesting that these drug treatments need only be given for a very short period around the time of scrapie infection by a peripheral route, suggesting that they influence some of the cellular events involved in determining the "speed" of the scrapie "clock". These early interactions between scrapie agents and host cells will clearly be of great importance in future studies of scrapie pathogenesis.

The physicochemical nature of scrapie agents is another fundamental question that needs to be answered. This has been an exceptionally difficult problem to study for several reasons—no one has consistently identified virus-like particles in scrapie preparations using the electron microscope, so we don't really know what kind of agent to look for and immunological and tissue culture methods are not available for measuring amounts of agent. This can only be done by infectivity assays which in mice are very time consuming because of the long incubation period. Also, no-one has succeeded in obtaining purified preparations of scrapie agent which would make characterisation so much easier. Many standard virological techniques are therefore inapplicable to scrapie research and somewhat indirect methods have been used, mainly based on studying the effects of various physical and chemical treatments on scrapie infectivity (Hunter, *Prog. med. Virol.*, **18**, 289-306; 1974). These studies have revealed a number of important clues.

In general, scrapie agents are very stable to many treatments which would be expected to inactivate most conventional viruses. This stability may be related to the intimate association of infectivity with cell membranes. The smallest membrane fragments with infectivity have a diameter less than 50 nm. Important studies by Haig, Alper and others of the effects of ultraviolet and ionising radiation suggest, however, that the scrapie-specific portion of infective membrane fragments (the portion that codes for different strains of scrapie) may be much smaller. Despite past controversy, there is no good reason why the scrapie-specific portion should not be nucleic acid, in which case the ionising radia-

tion data suggests that it may have a molecular weight of 150,000 daltons (about the same size as viroid RNA). Therefore the simplest working hypothesis suggests that the infectious scrapie agent is made up of a small specific nucleic acid intimately associated with membranes of the host cell. Implicit in this model is the concept that scrapie agents do not exist as discrete virions which can be easily separated from cell components.

Against this background it is interesting to read the paper by Cho and Greig on page 685 of this issue of *Nature* in which the isolation of 14 nm virus-like particles from mouse brain infected with scrapie agent is described. The authors have been very careful to state that "it is not known whether these particles are indeed the scrapie agent" which is an honest admission that so far there is no evidence of whether these particles have scrapie activity. This is an important point because experience in cancer research has taught us to be wary of accepting an aetio-

logical role for viruses isolated from diseased tissues. The central nervous system is a well known repository of persistent and latent viruses which may or may not be involved in disease. It is quite conceivable that the particles isolated by Cho and Greig represent a virus which is present in brain and may be activated by scrapie infection but which plays no role in the disease.

The crucial questions to answer are do these particles *per se* have high specific scrapie activity and can they be isolated from other tissues such as spleen and from other hosts infected with other strains of agent?

If the answer to both these questions is yes then the findings of Cho and Greig will considerably alter our thinking on the nature of scrapie agents but, more importantly, they will provide purified particles that will make physicochemical characterisation so much easier. If the answer is no, then the authors have apparently purified a virus-like particle which may still be of interest because of its small size. □

origin in the lateral growth zone. That is the conclusion Scholes has drawn from labelling DNA in the growing fish retina, where the label appears in synchronised waves in the red, or the blue, or the green cones but is never seen to spread laterally in the receptor layer, and so must presumably be travelling vertically.

Spatial frequency channels

Lamb's talk was followed by a scintillating review from Fergus Campbell (Cambridge University) on the psychophysical evidence for spatial frequency channels in human vision, which are not explicable at the receptor level. Horace Barlow, from the chair, raised the question of how to explain, at any level, how the Fourier analysis which Campbell has suggested as the basis for the frequency response can be combined with the retention of precise positional information. Campbell's response was to concede that the Fourier analysis must be done piecewise, so that much of the information in the frequency channels is positional and only some of it is broken down into spatial frequencies. As the physiological substrate for this dichotomy, however, he suggested that spatial frequencies might be coded in the large, fast-adapting Y fibres of the optic nerve and position in the slow X fibres. That remark elicited a fascinating case history from Geoffrey Arden, who has been using new television techniques for recording visual evoked potentials from multiple sclerosis patients. They can be shown initially to suffer the selective loss of their X fibres (*The Evoked Potential*, edit. by Desmedt, J., Oxford University Press, in the press), and they complain, not of the loss of detailed vision, but that they cannot tell the position of an object if it is moving.

Hard wiring in the cortex?

The mechanistic approach remarked by Wall at the BRA meeting is at its most impressive in the visual cortex, where, as Wall pointed out, the beginnings of abstraction are to be seen in the existence of cells which respond selectively to lines of a given orientation. A. J. Movshon (Cambridge University) briefly sketched the history of the controversy which still exists over the part played by postnatal experience in determining orientation selectivity, concluding with some arresting recent evidence in favour of it.

Movshon has followed up the experiments of Pettigrew and Blakemore on the reversal of cortical ocular dominance as a result of suturing first one eye and then the other eye of kittens during the sensitive period. By micro-electrode recordings across the cortex

More than meets the eye

by Miranda Robertson

The Brain Research Association Summer School, held in Cambridge on September 22-24, was followed by the Annual Meeting of the Institute of Biology, on the Physiology of Vision, in London on September 25 and 26.

THE dominant theme of both meetings was the interpretation of brain function in terms of the anatomy, physiology and psychophysics of its sensory systems. Patrick Wall, introducing a session on receptor systems at the BRA, remarked on a recent trend towards explaining perceptual phenomena purely on the basis of receptor mechanics, which he identifies as a resurgence of mechanism, traceable to Mountcastle at Johns Hopkins.

Photoreceptor interactions

The success of that approach at the sensory periphery was vividly illustrated by Trevor Lamb (Cambridge University), who explicitly related established psychophysical phenomena such as Weber's law and the Stiles-Crawford effect to the results of intracellular recordings from cones in the turtle retina (Baylor and Hodgkin, *J. Physiol., Lond.*, **242**, 729; 1974 and Baylor and Fettiplace, *ibid.*, **248**, 433; 1975). His concluding report was on electrical coupling between photoreceptors which at first sight seems

counter-adaptive since it degrades visual resolution. A prominent effect, and perhaps the function of such coupling is to reduce the intrinsic dark noise which apparently results from random opening and closing of the light-sensitive ionic channels in the photoreceptor membrane (Simon *et al.*, *Nature*, **256**, 661; 1975).

Lamb and his colleagues now have evidence in support of the notion that noise level in a single receptor depends on the extent of coupling to other cells. Very noisy cones turn out to have narrow receptive fields, whereas quieter ones respond to stimuli over much broader areas. Quantitative studies based on the degree of noise reduction are now under way to elucidate the pattern of coupling.

The question of receptor interactions arose again at the meeting of the Institute of Biology, where John Scholes (Kings College London) presented evidence for systematic (and probably antagonistic) interactions between red and green and green and blue cones in the retinae of rudd and goldfish, where the different photoreceptors can be distinguished morphologically. The same pattern of interaction is reiterated in the bipolar and ganglion cells fed by the photoreceptors; which makes it all the more fascinating that embryologically, the photoreceptor and its bipolar and ganglion cells all seem to have the same

of a series of these animals at different times before and after suture, he was able to follow the expansion and contraction of the columns as first one eye and then the other took control.

That process is not controversial, and has actually been photographed by D. Hubel (Harvard University) who showed some amazing slides of coronal sections of monkey visual cortex stained by the injection of labelled amino acid into one eye. The zebra stripes formed across the cortex by the ocular dominance columns were caught in the act of reciprocal contraction and expansion as a result of suture reversal, leaving no room for doubt about what was going on.

The disagreement arises over orientation selectivity, which Movshon and his associates claim is influenced by visual experience and Hubel and Wiesel claim is innately specified. Movshon's evidence comes from recordings of a few cells in reverse sutured kittens that could be stimulated from either eye. Such binocular cells in normal cats invariably have the same orientation selectivity for stimuli from right and left eyes. In the reverse sutured kittens, however, Movshon found a few binocular cells, presumably in the process of changing their ocular allegiance, which seemed at the same time to be changing their orientation preference. These cells were selective for different orientations when stimulated through the different eyes.

While that would seem to be compelling evidence for plasticity under the positive influence of experience in the cat, Hubel has begun to test the effects of stripe-rearing (in which visual experience is limited to bars of a single orientation) in monkeys and (in the one satisfactory specimen so far) has found no effect on the columnar pattern of orientation selectivity of cortical cells. Challenged by Movshon, he could offer no explanation for the discrepancies; nor could Movshon explain recent failures to replicate stripe-rearing results in cats.

Perceptual problems

Another embarrassment for visual neurophysiologists emerges from psychophysical experiments on stereopsis described by J. P. Frisby (University of Sheffield). According to the neurophysiology, stereopsis is mediated by binocular, orientation-selective cells which have a marked maximum response where the stimuli in either eye have a specific horizontal disparity on the retina. Frisby has tested depth perception in man (two men, himself and one other) using an adaptation of the Julesz random dot stereogram made with lines instead of dots. He has found that the perceived depth is a function of the length of the lines

Assessing prospects for Spacelab

from John Gribbin

A FEW months ago a series of experiments were flown on board NASA's CV-990 'flying laboratory' to simulate an orbital mission by the European Spacelab which is to be flown on the Space Shuttle during the 1980s. Some of the first scientific results from this ASSESS mission are presented in a series of papers commencing on page 649 of this issue, but the other lessons learned are also of great interest.

The joint ASSESS mission illustrated that a low-cost programme with a low level of preparatory requirements, testing and documentation can operate successfully under the proper management. Not only was the ASSESS mission itself fairly cheap (by space agency standards); it provided every indication that low-cost projects can work well when the real Spacelab gets off the ground, which must be encouraging news for many of the research groups working within tight budgets to try to take advantage of the opportunities which will be provided by the next generation of manned spacecraft.

And such aircraft simulations may have a significant role to play in developing instrumentation for the next generation of unmanned spacecraft as well. The ASSESS flights included an experiment from the Laboratory for Atmospheric and Space Physics (LASP) of the University of Colorado, a scanning two-channel spectrometer

which covered the wavelength range 270–650 nm and was a prototype of the ultraviolet spectrometer which LASP expects to fly on the forthcoming Pioneer Venus orbiter spacecraft. The mission provided a far better opportunity to sort out bugs in the system than any ground based operation, as well as producing valuable observations of Venus.

But ASSESS proved that there will be real problems in the practical operation of a flying laboratory. Human engineering of instrument control is necessary, as is a near real time high quality visual display of recorded data to confirm to the operator that successful measurements are being made. One clear result emerged from the human side of ASSESS. The best results come from operators who are trained scientists, understand the equipment and can use a soldering iron effectively on the spot to overcome the troubles they have diagnosed. There will, it seems, be no retreaded test pilots on the Spacelab, only a professional astronaut-pilot and the professional scientist-operators.

A second mission is now planned. NASA has indicated that the CV-990 will be available in October/November 1976, which would allow time for the data collected to be usefully incorporated into the final stages of the development of Spacelab.

used, a result which defies explanation within the current physiological paradigm.

No satisfactory physiological paradigm has in any case yet been proposed for perceptual phenomena which depend on activity beyond the primary visual cortex, a point implicit in the title to which Stuart Sutherland (University of Sussex) addressed himself: "There's more to vision than meets the eye." Sutherland believes in the use of computer programs as a testable way of representing how feats such as human visual perception might be achieved, and the first lesson to be learned from such programs is the enormous amount of knowledge and past experience people bring to bear on seeing things (consider the difference between a layman and a trained histologist, looking at a microscope slide). The lesson from the current lumbering knowledge-based computer vision programs however seems to be that brains have some way of leaping to global conclusions while computers are still creeping along line by line. □

Protoplasts at Nottingham

from E. C. Cocking

The 4th International Meeting on Yeast and Other Protoplasts was held at Nottingham, UK on September 8–12. The proceedings will be published by Academic Press.

THIS was one of the rare occasions when workers on bacterial, fungal and higher plant protoplasts came together to exchange viewpoints. As a result it was possible to compare the properties of protoplasts from both eukaryotic and prokaryotic systems; and for many of the participants this was the major attraction.

Enzymatic proceedings for the degradation of the cell wall, resulting in protoplast release, are now largely standard procedures; and, although the enzymes used for isolation differ in the various systems, the influence of the exact nature of the cell wall was

central in all the discussions of protoplast isolation. The bacterial membrane was shown by R. E. Marquis and T. R. Corner (Rochester University, New York) to be a remarkably extensible structure, while the membrane surrounding fungal protoplasts attracted the attention of D. Kerridge *et al.* (Cambridge University) to ascertain whether difference in chemical composition of plasma membranes, from different growth stages in the growth cycle, could be correlated with differing sensitivities to polyenes.

The activity of the plasma membrane in the synthesis of a new cell wall around isolated protoplasts was described in detail for all three protoplast systems. The induced, heritable, condition of wall-less-ness in bacteria was shown not to be due to alteration in the DNA. No comparable stable L forms have been found to arise from fungal protoplasts, or from higher plant protoplasts. But Y. Meyer *et al.* (Heidelberg University) described special conditions under which higher plant protoplasts regenerate a non-rigid, polysaccharide containing, "pseudo-wall". The photosynthetic activity of plant leaf protoplasts was very lucidly described by G. E. Edwards *et al.* (University of Wisconsin). It is readily possible to compare the photosynthetic activities of protoplasts isolated from C3 and C4 plants. Indeed, such protoplasts are more useful than isolated chloroplasts for certain photosynthetic studies, since enzymes of the cytoplasm, peroxisomes and mitochondria may be involved in carbon metabolism during photosynthesis.

As might be expected, great interest centred on the activity of plasma membranes in the fusion of isolated protoplasts. Fusion of bacterial protoplasts is still neglected; work on the polyethylene glycol induced fusion of fungal protoplasts, is however, actively continuing, and L. Ferenczy *et al.* (Szeged University) and J. Anné (Louvain University) and J. F. Peberdy (University of Nottingham), were able to show how fusion was expressed by heterokaryon formation between nutritionally complementing strains. Until recently, the only means of hybridisation in plants was by sexual crossing; E. C. Cocking (University of Nottingham) surveyed the ever increasing momentum of work on an "alternative to sex" which involves the induced fusion of somatic plant protoplasts, and, following adequate selection and culture, the regeneration of hybrid plants; the importance of combining this somatic hybridisation with the sexual genetics of higher plants was highlighted. Following the report earlier this year of the induced fusion of yeast protoplasts with hen erythrocytes, con-

siderable interest was aroused by the report of M. R. Davey and J. B. Power (University of Nottingham) of ultrastructural studies which showed that the induced fusion of the yeast plasma membrane with that of higher plant protoplasts takes place within uptake vesicles, following treatment of mixtures of these protoplasts with polyethylene glycol.

F. E. Young *et al.* (Rochester University) showed how the bacterial surface plays an extremely important role in the uptake of DNA and adsorption of viruses. In the eukaryotic fungal and higher plant systems comparable studies are at a much earlier stage of development, and there are many technical difficulties still to be resolved. It was encouraging, therefore, that D. Hess (Hohenheim University) was able to show that there is a considerable enhancement of DNA uptake when the DNA is fed as a calcium phosphate complex. Also encouraging were reports from several laboratories that polyethylene glycol may be a useful agent in attempts to establish endosymbiotic associations between higher plant cells and microorganisms *in vitro*. Those more physiologically orientated workers, interested in cell fractionation, noted with interest the report of T. Boller *et al.* (ETH, Zurich) of the use of the polybase, DEAE-dextran, for the isolation of vacuoles by the lysis of yeast protoplasts.

There is no doubt that this meeting fulfilled a long felt need for greater contact between those working on prokaryotic and eukaryotic systems. When the next International Meeting is held in Szeged, Hungary, in 1978 it is intended to extend further this induced fusion of protoplast disciplines.

Problems in the derivation of mRNA

from a Correspondent

The third Arolla workshop on the formation of messenger RNA in eukaryotic cells was organised by Klaus Scherrer at Arolla, Switzerland from September 2-6.

As on the two previous occasions, the main focus was on mRNA synthesis, but related topics were included. The subject matter was therefore gene expression in the round.

The meeting opened with reviews of chromatin structure and the characterisation of nonhistone proteins. Despite universal agreement that chromatin has a beaded structure, containing discrete protein clusters, there is still considerable doubt both as to the organisation of the clusters and on the relationship

between the histones and DNA. Histone H1, which shows microheterogeneity and a varying mass ratio to DNA, is not integrally involved in the structure of the beads, which appear to be octamers containing two molecules each of histones H2A, H2B, H3 and H4. The authenticity of reconstituted preparations of chromatin remains in some doubt, since they do not generate the array of fragments containing multiples of 200 base pairs of DNA which is characteristic of mildly digested native chromatin. Whether reconstituted preparations can serve as accurate templates for transcription *in vitro* remains controversial.

The nonhistone proteins comprise a dauntingly large number of species which vary in molecular weight from less than 10,000 to more than 200,000 daltons. There was no indication of any hope that the problem of defining these proteins is nearer solution. The observation that 20% of the proteins bound to DNA in low salt are removed below 0.2 M and 20% above 3 M may help to explain conflicting views on the metabolic stability of nonhistone proteins. Progress has been made in identifying increasing numbers of prominent nonhistone protein components, including DNA ligase, DNA polymerase, histone kinase, histone methylase, actin, myosin, tropomyosin and α and β tubulins. But while this is valuable information in its own right, we cannot seriously hope to identify the putative regulatory proteins by a process of elimination.

Discussion of satellite DNAs *per se* gave the impression that progress in this area is now slow, perhaps inevitably, since the structure of the satellites is by now understood quite well while their possible functions are much less amenable to investigation. But a satellite-like structure of small scale tandem repetition has been observed in the nontranscribed spacer of ribosomal DNA, which displays microvariation in length. One possible function for the spacer in this system may be to help maintain the homogeneity of transcribed DNA sequences by increasing the frequency of unequal crossing over. Another tandemly repeated DNA sequence on which rapid progress has been made is, of course, the histone DNA. There appears to be a basic repeat unit which contains the sequences for all five histones, separated by short spacer regions. Whether a single precursor for all the histones is transcribed is not known. Progress with both rDNA and histone DNA has clearly received a tremendous boost from the successful cloning of the DNA by means of bacterial plasmids.

Bridging the gap between DNA organisation and chromatin structure on the one hand and on the other the

characterisation of the primary transcription product, there was a summary of recent electron micrographic observations on the active interphase chromatin of *Drosophila* nurse cells. This work has generated the first description, in these terms, of nonribosomal transcription units. In most cases, when the lengths of RNP fibres were plotted against distance along the DNP axis, a clear correlation extended over lengths averaging 3.2 μm . One appealing interpretation of these results is that each observed transcription unit is equivalent to a single cistron as defined by genetic analysis, with an apparent average length of 6 μm . These results contrast with biochemical observations of much smaller nuclear RNA molecules, a paradox whose resolution may help define the derivation of mRNA.

The existence of a giant nuclear precursor of mRNA remains controversial. Work on duck erythroblasts, mouse Friend cells, hen oviducts and L cells was reported; but there is no clear consensus on the characteristics of any precursors. The failure of ten years of effort to solve this problem suggests strongly that new techniques may be needed, perhaps for example the use of plasmid clones of appropriate sequences. Work in one system stands out, however; it was reported that only 20–25% of the total 75S product of Balbiani ring 2 of *Chironomus* salivary glands ever reaches the nucleoplasm and only 5% enters the cytoplasm. If this does not merely reflect the sickness of explanted glands, it would suggest a powerful processing component in gene expression.

In a session devoted to the ends of eukaryotic mRNA molecules, some possible functions emerged for 3' poly(A) sequences and 5' methylated caps. If the length of poly(A) on globin mRNA is reduced to less than 40 residues, its decay after injection into oocytes is accelerated. Decay is very rapid when the length is less than 15 residues. The validity of this observation is supported by the fact that during the later stages of reticulocyte maturation the length of the poly(A) of the mRNA that can be recovered remains a constant 40–50 residues, despite a net fall in the amount of mRNA per cell. Viral mRNAs which normally are capped require methylation of the 5' terminal guanine for translation in the wheat germ system. In this system methylation is required for formation of the initiation complex, and from this a 35 nucleotide 5' terminal fragment can be isolated following treatment with ribonuclease. Picorna viruses, which normally are not capped, are not translated in this system and are translated without methylation in others.

Much progress has been made in sequencing studies. Partial sequences of several purified poly(A)-containing mRNAs now are available; in several cases it is clear that there is a significant length of nontranslated RNA at each end, longer at the 3' end. Sea urchin histone messengers also contain a nontranslated region. Rabbit α and β globin mRNAs and a mouse immunoglobulin L chain mRNA contain a 3' terminal sequence which suggests the existence of two hairpin loops which may or may not be stable *in vivo*. If the loops are present, these three messengers contain the same short sequence along the spine of the molecule. Hen ovalbumin mRNA does not contain this sequence. But the tetranucleotide adjacent to the poly(A) moiety is the same in all four messengers; this might be part of signals concerned, for example, with termination of transcription or polyadenylation.

The inherent tendency of hnRNA to form aggregates makes doubtful many of the conclusions based on fractionation by means of affinity columns, since these are often operated under conditions likely to promote aggregation, and so molecules with no affinity might be aggregated with those that have and thus be retained. A promising approach to the structure of hnRNA appears to be the analysis of the smaller sequences obtained by fingerprinting T_1 digests of total hnRNA and analysis of the small (3%) residue which is resistant to ribonuclease A. Sequence analysis of the ribonuclease-resistant hairpins suggests that many may have the same or a similar sequence. This family of sequences has some 10^5 members, each perhaps 150 bases in length, and should correspond to something less than 1% of the mammalian genome. A fingerprint pattern containing some characteristics of the ribonuclease-resistant RNA is obtained from both the hnRNA and mRNA which hybridise with DNA at very low C_{ot} values. There were indications of a relationship between the RNase-resistant hnRNA hairpins and the S1-resistant foldback loops of DNA. The distribution in CsCl gradients of the DNA sequences complementary to hnRNA hairpins is different from the bulk DNA distribution, which would be consistent with some clustering of these sequences in DNA.

Since no enzymes which cleave RNA are known in eukaryotes attention was focused on those found in bacteria, where it seems that several different classes of recognition process are involved. Five hexanucleotide sites on RNA cleaved by RNase P are all different. And mutational changes in different parts of the tRNA precursor affect its processing; some of these are near the cleavage site, some are in the

complementary part of the molecule, and some are quite remote. On the other hand, the known 5' termini of T7 RNA fragments all have very similar sequences. One general point of obvious importance is the observation that hairpin loops are invariably implicated in bacterial processing reactions. A rather striking observation is that RNase D (=Q=III) can cleave 45S ribosomal precursor to yield a molecule sedimenting at 28S; however, characterisation of the product clearly is necessary before this can be interpreted. A feature of this enzyme is that it preferentially cleaves at specific sites in low salt, but preferentially solubilises double-stranded RNA in high salt; this reinforces the view that the products of its action must be carefully characterised. □

Dale centennial celebrations at Cambridge

from L. L. Iversen

The Sir Henry Dale Centennial Symposium—Post-synaptic Actions of Neurotransmitters was held in Cambridge on September 17–19.

In the days when Sir Henry Dale made his pioneering contributions to the understanding of the actions of histamine, adrenaline and acetylcholine (ACh) on various tissues including those of the nervous system, he deliberately avoided the use of the term "receptor", then used in a purely operational sense. Such reticence is no longer necessary, as was amply demonstrated by the up-to-date reviews of drug-receptor interactions presented at the symposium.

The highest development of such studies was described by J. P. Changeux (Pasteur Institute, Paris), reviewing the work of his group on the purification and characterisation of the nicotinic cholinergic receptor protein from excitable membranes of the electric organs of fish. Changeux described a model for the action of the ACh receptor which postulates the existence of the receptor in three possible states: resting, activated and—after prolonged exposure to ACh or a related agonist—a desensitised condition. Desensitisation can be demonstrated *in vitro* using membrane fragments exposed to carbachol, and results in binding of ACh and other agonists with unusually high affinity, but without transduction into a change in membrane sodium permeability, as in the normal transition from resting to activated states.

Remarkable new refinements in the neurophysiological study of ACh at the

neuromuscular junction were reported by Sir Bernard Katz (University College, London) and S. Kuffler (Harvard Medical School). Katz described how statistical analysis of the increased muscle membrane noise generated on exposure to ACh has shown the precise characteristics of the changes in membrane conductance elicited by ACh in individual membrane ionic gates or channels. Kuffler described work by his group on snake muscle, in which it is possible to apply measured amounts of ACh directly to the endplate areas of muscle membrane from which the nerve terminal has been removed by microdissection. They have established that less than 10,000 molecules of ACh are needed to mimic the effects of one quantum of transmitter normally released by the nerve terminals (several hundred such packages are released by each nerve impulse).

A. S. V. Burgen (National Institute for Medical Research, London) described rapid progress in characterisation of muscarinic cholinergic receptors. The binding characteristics of labelled antagonist drugs to muscarinic receptors in smooth muscle or brain correspond well to those predicted from previous pharmacological experiments, but those of agonists seem more complex. Graphs of agonist binding followed non-linear Hill plots, suggestive of complex cooperative interactions with the muscarinic receptor sites, or, more probably, of the existence of more than one category of agonist binding site. The latter possibility is in accord with the earlier findings of Spero and Burgen, that agonist effects on ion fluxes and contraction in smooth muscle are not always parallel. When a covalent alkylating reagent for muscarinic sites is used, N-propylbenzilylcholine mustard, the muscarinic receptor molecule in smooth muscle can be identified as a polypeptide with a molecular weight of 85,000.

P. Greengard (Yale University) reviewed evidence that the post-synaptic actions of ACh at muscarinic receptors and of other transmitters, notably noradrenaline and dopamine in the brain, may be mediated by changes in the concentrations of cyclic AMP or cyclic GMP in the post-synaptic cells as a result of activation of a transmitter-sensitive adenylate or guanylate cyclase in the synaptic membrane. Recent work supports the hypothesis that cyclic nucleotides may produce synaptic potentials by activation of a protein kinase that in turn alters the level of phosphorylation of proteins in the synaptic membrane and consequently the ion permeability characteristics of the cell. In synaptic membranes from brain, cyclic AMP activated the phosphorylation of a few specific membrane proteins, some of which have

been purified. In a simpler system, the turkey red blood cell, which responds to catecholamines by changes in ion permeability through a cyclic AMP mediated β -adrenoceptor mechanism, it was possible to show a good correlation between the extent of increased phosphorylation of a specific membrane protein and the associated changes in intracellular cyclic AMP and ion fluxes caused by exposure to catecholamines.

Experiments on dopamine receptors in mammalian brain, using radioactively-labelled dopamine or the antagonist drug haloperidol as ligands, were described by S. Snyder (Johns Hopkins University). The data on the direct interaction of agonists and antagonists with CNS dopamine receptors complemented those of Greengard and others from studies of drug interactions with the dopamine-sensitive adenylate cyclase in mammalian brain. As with several other transmitter and drug receptors, there was evidence that dopamine receptors could exist in two stable configurations, one with a high affinity for agonists, and the other with lower affinity for agonists but a high affinity for antagonists.

D. Jenkinson and J. Black (University College, London) discussed the dual receptor systems that mediate adrenaline and histamine responses in the body, the α - and β -adrenoceptors, and H1 and H2 histamine receptors. The use of radioactively labelled β -adrenoceptor antagonists, such as alprenolol, allows the ligand binding approach to be applied here also. The possible interrelations between the two types of adrenoceptor remain tantalising but obscure; Jenkinson's own group has recently shown that β -adrenoceptor agonists could elicit changes in ion fluxes in isolated tissues only after a previous exposure to α -adrenoceptor stimulation.

G. Aghajanian (Yale University) described the work of his group on the neurophysiological characterisation of receptors for 5-hydroxytryptamine (5-HT) in mammalian brain. The actions of 5-HT on receptors located on the surface of the 5-HT neurones themselves are very potently mimicked by *d*-LSD and related hallucinogens, which thus cause a powerful inhibition of the firing of 5-HT neurones in the brain.

Surface bound antibodies on B lymphocytes and on mast cells have many similarities to the receptor macromolecules of excitable tissues, and M. Raff (University College, London) reviewed the work of his group on the unexpected lateral mobility of such ligand-binding proteins in the cell membrane. Recent fine structural studies of histamine release from mast cells throw new light on the mechanism of exocytosis in which cell membrane protein seems to be conserved by migration from

regions of contact between secretory vesicles and plasma membrane.

Finally J. Axelrod (National Institute of Mental Health) reviewed work by his group on the longer term changes that are initiated by transmitter molecules impinging on post-synaptic tissues. In adrenergic neurones and in the pineal gland the extent of synaptic stimulation controls the rate of synthesis or degradation of key enzymes in the biosynthesis of catecholamines or the pineal substance melatonin. The pineal gland in particular offers an elegant model system for studies of the rapid and complex changes that can occur in protein synthesis, enzyme activity and the sensitivity of the β -adrenoceptors to stimulation by catecholamines. Changes in receptor sensitivity leading to supersensitivity (with decreased stimulation) or subsensitivity (on prolonged stimulation) can occur in this tissue within a few hours and are associated with parallel alterations in the number of β -adrenoceptors detectable by radioactive alprenolol binding studies. □

Crystallographers in Amsterdam

from Olga Kennard

The 10th International Congress of Crystallography was held in Amsterdam on August 7-15.

AMSTERDAM in the heat wave, with flags flying, sunlight glittering on the canals and 1,500 crystallographers gathered for their 10th Congress—29 years after the foundation of the International Union of Crystallography and some 63 years since the beginning of X-ray crystallography. A young discipline—and even the disciples have hardly aged. The pace of the Congress was set by Paul Ewald, now 87, who not only gave a memorable discourse, but who, by his penetrating questions, made many a younger contributor re-examine his basic assumptions.

Crystallography is still full of excitement and experimentation. Experimentation even in scientific communication, which at Amsterdam meant great emphasis on poster sessions held in specially constructed booths complete with tables, chairs and blackboards—veritable psychiatric clinics! Altogether some 600 contributors opted for posters compared with 300 short oral papers. The sense of "discussions in corridors" prevailed in the booths as shown by the continuous and spirited arguments which otherwise so rarely develop in lecture rooms after short presentations.

Poster sessions were complemented

by invited lectures. Some such as Phillip Coppens's (State University of New York, Buffalo) review of the accurate determination of electron densities, drew together and made coherent sense from a scatter of individual exhibits and gave the necessary background and critical evaluation for the non-specialist. More of these link-lectures would ensure even greater effectiveness of the poster sessions at future Congresses.

The eight general lectures were all delivered with great style and high information content. Caroline MacGillavry's discourse on "Order and Beauty" continued the old crystallographic tradition of linking the two cultures. A. I. Kitaigorodski's (Institute of Elemento-Organic Compounds, Moscow) talk on "Interaction in molecular and non-molecular crystals and lattice dynamics" should perhaps be mentioned for its simplicity of approach, which set a good example, especially to the younger generation of computer-bred crystallographers juggling with too many variables. His definition of scientific theory: first rate if it can predict, second rate if it can forbid and third rate if it can explain "post factum", became a useful yardstick for the Congress.

There were 27 main topics covering all aspects of crystallography from perfect crystals to almost total disorder. Macromolecules, both globular and fibrous, were well represented, with an impressive exhibit of models, and considerable emphasis on the functional and evolutionary aspects. There seems to be an imminent breakthrough in virus structures and a particularly exciting account was given by John Champness (MRC Laboratory of Molecular Biology, Cambridge) on the structure of the disk of tobacco mosaic virus protein to 5 Å resolution.

It would be tempting for this rapporteur to discuss small biological molecules, for example the first phospholipid and membrane component structures (R. Mason, University of Sussex; S. Abrahamsson, University of Göteborg; and M. Sundaralingam, University of Wisconsin, Madison) but invidious if some fascinating compounds in the six other structural sections are neglected. The general impression was that crystallographers have now overcome their sense of guilt at the comparative ease with which structures can be solved and are turning their attention to a more fundamental interpretation of the results in terms of physicochemical properties, function or mode of action.

Not all structures yield to current techniques and one of the highlights of the meeting was the session on direct mathematical methods. H. Hauptman (Medical Foundation of Buffalo) set the

theme with a general talk which included the new quartet invariants—a true breakthrough! P. Main (York University) discussed the use of known chemical information and his matrix approach looks very promising.

B. Busetta (University of Bordeaux) applying these methods, reported the solution of perhaps the largest "small" structures to date with 116 and 126 light atoms in the asymmetric units of difficult, non-centric space groups. Rumour has it, however, that the Karles (Naval Research Laboratories, Washington) still keep the record, with a modification of valinomycin (156 atoms in space group P1). These new developments offer real promise to view, without recourse to heavy atoms, the structure of compounds perhaps as large as 3,000–5,000 daltons, at atomic resolutions.

An interesting feature of the exhibits was a structure-solving clinic using a minicomputer (G. Sheldrick, Cambridge University; E. Oeser, Technische Hochschule, Darmstadt). Sixteen apparently insoluble structures were submitted and six were actually solved and refined during the Congress. Need sponsors have any better proof of the value of international meetings!

A final word about the small community of Dutch crystallographers. They organised the Congress with imagination (the brass band of the Amsterdam Police instead of welcoming speeches!) and a friendly efficiency which ensured the highest possible satisfaction quotient—both scientific and social—for all participants. □

Vaccination against fertility

from a Correspondent

The Third International Symposium on the Immunology of Reproduction was held in Varna, Bulgaria on September 21–25. It was supported by the Union of Scientific Workers in Bulgaria, the G. Dimitrov Agricultural Academy, the Bulgarian Academy of Sciences, the Ministry of Health in Bulgaria and the World Health Organisation. The proceedings will be published by the Bulgarian Academy of Sciences.

THE proceedings in Varna revealed good prospects for an anti-fertility vaccine, potentially invaluable in the battle against world overpopulation. Of particular interest were the initial findings of G. P. Talwar (All India Institute of Medical Sciences, New Delhi) who injected a few women with the specific portion (the beta subunit) of human chorionic gonadotropin

conjugated with tetanus toxoid. The antibody titre to the gonadotropin rose to high levels which were maintained for more than a year, and the antibody was shown to inhibit the biological activity of the gonadotropin, which plays an essential role in pregnancy. In one case, a booster inoculation was given when the antibody titre fell and this was followed by a return to former levels. Tests on a wide range of bodily functions provided no evidence of any undesirable side-effects of the treatment. The women had previously requested surgical sterilisation and menstrual cycles continued throughout the period of observation. Similar studies to Talwar's are being initiated in several institutions in Europe and the Americas, so that thorough assessment of the safety of the method should be accomplished in the near future. Large-scale clinical trials will follow if indications are favourable. In spite of Talwar's apparent breakthrough, however, detailed studies are continuing in various laboratories on the synthesis and biological activity of amino acid chains representing parts of the non-homologous region of the beta subunit which could prove highly specific antigens. The possibility of immunisation with other specific placental proteins is also being pursued actively, chiefly with components identified as SP1 and PP5.

Probably the largest single topic of the symposium had to do with the antigenic structure of spermatozoa and their immunological potential in both the male and the female. In the human male the concern was primarily with infertility associated with anti-sperm antibodies in the circulation, and with the risk of autoimmune damage in vasectomised men. The impressively extensive use of vasectomy in India was described by S. S. Rao (Institute for Research in Reproduction, Bombay) who noted that many subjects were found to have high titres of circulating antibody apparently without serious consequence. The prospects of developing a contraceptive vaccine for males as an alternative to vasectomy has been improved by the use of adjuvants with less drastic local effect than Freund's adjuvant. With the female the emphasis is on the control of fertility by immunisation with spermatozoa or specific sperm antigens, such as hyaluronidase, acrosin, LDH-X and antigen T. Progress in this field was usefully reviewed by W. R. Jones (Flinders University of South Australia, Adelaide). Antibodies against these antigens do not cross-react significantly with somatic tissue antigens, although human acrosin is very similar in amino acid composition to pancreatic trypsin. Some depression of fertility is achieved by active immunisation but the results are not yet

satisfactory. Curiously enough the reduction in fertility produced by anti-LDH-X antibodies is owing mainly to embryonic death during cleavage and implantation. The fact that sperm-agglutinating but non-immobilising sera could prevent sperm penetration through cervical mucus in an *in vitro* test was reported by B. Boettcher (University of Newcastle, New South Wales). S. Shulman (New York Medical College) noted the occurrence in cervical mucus of sperm-immobilising antibodies active in the absence of complement. S. Isojima (Hyogo Medical College, Nishinomiya) described an improved microtechnique for detecting sperm-immobilising antibodies in cervical mucus. The risks involved in the use of placental hormones and sperm antigens were considered critically by K. S. K. Tung (University of New Mexico, Albuquerque).

The foetus as a homograft received its share of attention, the feeling clearly being that the reasons for the lack of foetal rejection are still poorly understood. But further support was provided for the idea that genetic dissimilarity favoured implantation. □

Charting the cell cycle

from M. Vicente

The Third European Workshop on the Cell Cycle sponsored by the European Cell Biology Organisation was held in Edinburgh on September 23-25.

SIMPLE systems still seem to be leading the race for the understanding of the events constituting the cell cycle. Although the majority of the papers dealt with eukaryotic systems, the most interesting results were reported from studies of prokaryotes or relatively simple eukaryotes such as yeast and the slime mould *Physarum polycephalum*. These organisms share the property of being either single cells or plasmodia in which the life history of a growing individual is not heavily disturbed or dependent on the presence of other individuals or their metabolic products. Moreover all of them are suitable for biochemical and genetical studies.

M. Masters (University of Edinburgh) has produced strains of *Escherichia coli* in which a duplicate copy of the chromosomal origin of replication is carried on a plasmid. Such strains have a significantly reduced number of chromosomes per cell mass and also divide at a proportionally larger size. These properties are consistent with the idea that there is a fixed amount of some substance required for the initiation of DNA replication at the chromo-

some origin and that the presence of extra copies of this origin results in competition between the chromosome and the plasmid for initiation at each mass doubling.

Three communications by P. Thuriaux *et al.*, P. Fantes and P. Nurse (University of Edinburgh), gave a new view of the cell cycle in the fission yeast *Schizosaccharomyces pombe* obtained from the study of mutants in which progression through the cycle is blocked or altered at different stages. There is a close similarity between the cell cycle in *Saccharomyces cerevisiae* and that in *S. pombe* as described by Thuriaux *et al.* Both cycles are in turn very similar to the *E. coli* cycle summarised at the meeting by W. D. Donachie (University of Edinburgh). The cycles in the three species have two independent pathways, one involving chromosome replication, both of which are required for the normal progression of the cell towards division. There is also evidence suggesting that the pathways comprise several sequentially dependent reactions.

Homeostatic control of cell size in *S. pombe* was observed by P. Fantes studying a thermosensitive mutant blocked at a stage before nuclear division at the restrictive temperature. The treatment does not seem to disturb cell growth in other ways and allows cell elongation to proceed. In these circumstances a negative correlation between the length of a cell at division and the duration of the following cycle is observed once the cells are shifted back to the permissive temperature. Some of the processes leading to division are more dependent on time than on size, however, as no matter how long the cells become before the temperature release they always require a minimum time before they can divide.

That initiation of DNA synthesis can be controlled by size while nuclear division is controlled by time was suggested by Nurse when interpreting his observations in a *S. pombe* mutant in which mass at division is half the wild type value and DNA synthesis is displaced to a later stage of the cycle while timing of nuclear division remains unchanged. The simultaneous functioning of timing and size controls is one more aspect in which the cell cycle in *S. pombe* is analogous to that of *E. coli*.

Replicating DNA fibres of *S. cerevisiae* have been made visible by autoradiography, after pulses of radioactive uracil, by D. H. Williamson and T. D. Petes (National Institute for Medical Research, London). They find that DNA replication proceeds bidirectionally in most of the replicons starting from several replication origins in each fibre, as in other eukaryotes. Therefore most of the replicons seem to initiate synthesis very early in the S period as

replication becomes resistant to inhibition of protein synthesis once the S period has started.

A quite different replication pattern is present in *Physarum polycephalum* in which several protein-dependent initiation events are detected by cycloheximide inhibition. Studying replication of DNA during the step that takes place 30 min after mitosis, F. Haugli (University of Tromsø) has isolated, after short pulses of ³H-thymidine, 7S DNA fragments very similar to the *E. coli* Okazaki pieces. These fragments are gradually chased into longer intermediates by a process that closely resembles the joining by ligase in *E. coli*.

Both R. Braun and V. M. Vogt (University of Berne) and H. W. Sauer *et al.* (University of Konstanz) suggested that the DNA that codes for ribosomal RNA in *Physarum* is contained in a structure that resembles morphologically a bacterial plasmid. In addition Braun and Vogt have found that replication of this rDNA occurs at random during G₂, a mode of replication that is consistent with that found in plasmids.

Reports on *Physarum* presented at the workshop show that studies on this organism are in a state comparable with that of *E. coli* in the early 1960s—that can be taken optimistically or pessimistically depending on how one views it. □



A hundred years ago

SIXTH REPORT OF THE SCIENCE COMMISSION

THREE times within the last twelve years a Royal Commission has reported on the science teaching of our higher schools. In 1864 the Public Schools Commission announced that from the largest and most famous schools of all it was practically excluded. In 1868 the Endowed Schools Commission declared that the majority of school teachers had accepted it as part of their school work. The Science Commissioners of 1875, in their Sixth Report, on Science Teaching in Schools, testing this statement by inquiry, reports that of 128 endowed schools examined by them not one-half has even attempted to introduce it, while of these only 13 possess a laboratory, and only 10 give to the subject as much as four hours a week. And this statement is curiously illustrated by the statistics of the recent Oxford and Cambridge School Examination, which show that out of 461 candidates for certificates from 40 first-class schools, while 438 boys took up Latin, 433 Greek, 455 Elementary Mathematics, 305 History; only 21 took up Mechanics, 28 Chemistry, 6 Botany, 15 Physical Geography.

From *Nature*, 12, 549, October 28, 1875.

articles

Simulating experiments for Spacelab

In June this year a number of flights were made in a NASA CV990 aircraft to evaluate designs for experiments to be carried aboard Spacelab from 1980 onwards. Here five of the groups involved describe what they achieved. NASA and the European Space Agency recently decided to carry out another similar mission next year.

Measurements of ozone and minor atmospheric constituents

THE instrument flown was an absolute spectrometric radiometer for the spectral range $3\text{--}40\text{ cm}^{-1}$. Such an instrument on a space platform would be used to measure the cosmic background spectrum and to monitor atmospheric constituents. At aircraft altitudes the atmospheric emission prevents the first, and the scientific objectives set for the flights were therefore to explore the spectral assignments and concentrations of ozone and minor constituents of the atmosphere and in so doing to test developments of technique. Previous experiments had shown the need for improved techniques that would give a resolution of at least 0.01 cm^{-1} unapodised so that overlapping emission lines from different constituents are resolved, that would allow an interferogram to be recorded in no more than a few minutes because significant changes can occur in atmospheric conditions in such time intervals, and that would give measurements of emission in absolute terms in order to make possible quantitative estimates of constituents. The changes required in the instrumentation¹ to give these improvements were the introduction of a fast data acquisition system, the adoption of the polarised interferometric method both to suppress the effects of signal fluctuations and to allow continuous reference to a cooled black-body calibration source^{2,3}, and the incorporation of greatly improved germanium bolometric detectors (P. A. R. Ade and S. El-Atawy, personal communication).

At the altitude of the aircraft (up to about 39,000 feet) there was appreciable atmospheric water vapour. Nevertheless, most

of the spectra were recorded at an elevation of no more than 14° to give a long path for the minor constituents. The aircraft was, during most flights, close to the tropopause (high at that time of the year) and it would have been unproductive to take measurements over a range of elevation angles so as to give limb-scanning information on the vertical structure of the atmosphere (though a suitable input mirror system had been incorporated for this purpose). It was, however, essential to have a servo-controlled input mirror, taking its drive from the aircraft's inertial guidance system to maintain constant elevation during the recording of an interferogram.

The emission was continuously compared with that from a black-body cavity; to give absolute calibration two black-body cavities were used, one at ice temperature and the other at liquid-nitrogen temperature. More than 100 interferograms were recorded, and even though the analysis is not yet complete it is clear that the new techniques were successful. To illustrate this, Fig. 1 shows small parts of two of the emission spectra, relative to the ice black body. The interferogram for each was recorded in 8 min and the spectrum obtained covers the range $3\text{--}40\text{ cm}^{-1}$, of which only the part from 20.5 to 22.5 cm^{-1} is shown. The Fourier transform was linearly apodised to give a resolution of 0.02 cm^{-1} . One of the spectra is displaced upwards to avoid overlapping. Both were obtained at an altitude of 33,000 feet and at an elevation angle of 14° ; they were recorded successively. It can be seen that most features are reproduced in the two spectra. The positions of lines of possible constituents of the atmosphere are marked at the top of Fig. 1, as derived from theoretical calculations and laboratory measurements (J. W. Fleming, private communication).

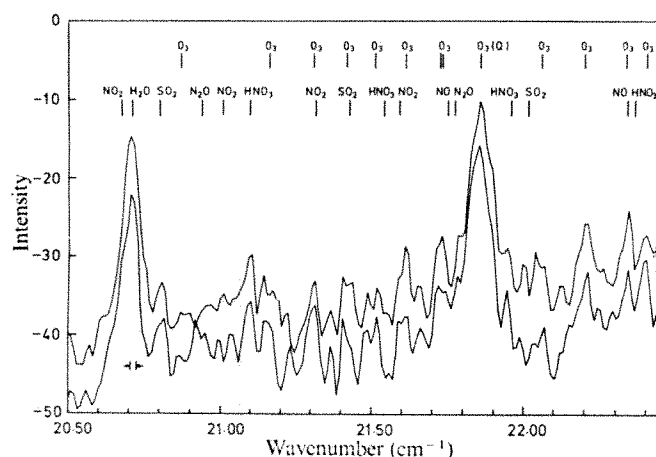
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Fig. 1 Parts of two of the emission spectra.



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Photography and photometry of the near infrared OH airglow

WE are able¹ to photograph the near infrared OH airglow, and the ASSESS mission allowed us to carry out: (1) horizontal parallax measurements of airglow features over a wide geographic range, using the plane's motion to generate a baseline; (2) photometry over a wide range in latitude and longitude to



Fig. 1 1-s exposure at 9:15 UT, June 18, 1975 at about 39,000 feet over Oregon.

study any geographical variation in intensity; (3) correlation studies of the brightness of OH bands between 690 and 840 nm for comparison with earlier work².

Our instruments were modified for aircraft use primarily by automating the repetitive measurements and incorporating image intensifier tubes to reduce exposure times, thereby minimising smear of photographs due to aircraft roll.

Three separate instruments were flown. (1) A 35-mm camera obtained exposures every 1 min of a $25^\circ \times 35^\circ$ area of the sky. A two-stage image tube with extended S-20 photocathode, 50-mm focal length input lens, and 720-nm cut-on filter, provided usable photographic densities in 1 s. (2) Colinear with the 35-mm camera an absolutely calibrated photometer viewed the central portion of the picture. An extended S-20 phototube preceded by a 7.5 cm $f/5$ telescope and an eight-position filter wheel with 20-s dwell time comprised the photometer. Four 10-nm bandpass filters were centred on OH bands at 690, 730, 790, and 840 nm, two filters were centred on non-airglow regions at 710 and 820 nm, one filter was identical to that on the 35-mm camera, and one position was opaque so that the dark current could be measured. (3) A 16-mm movie camera photographed another image tube with input lens and filter every 2 s, giving a continuous record of aircraft roll, as well as providing a motion picture of the passing airglow structures, tropospheric clouds, stars, and the Milky Way.

During the ASSESS mission 18 h of data (representing about 97% of the time suitable for airglow studies) were obtained. The entire mission of 14 flights has provided some 3.5 h of data per flight.

We have 2,500 35-mm frames (see Fig. 1), plus 45 h of photometer data, to analyse. With these data we will study the variation of parallax emission heights with geographical location and the relationship of OH patch or stripe size to emission height. The photometer will show any correlation between OH band intensity and geographical position, as well as the correlations between band intensities. By comparing photographic and photometric data, a correlation will be sought between type of structure and/or intensity with the height of the tropopause, magnetic and/or solar activity indices, local weather, and geographical features such as mountain ranges.

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Filter wedge spectroscopy in the region 3–6 μ m

SECTRA of Venus obtained previously in the region 1–6 μ m led to the identification of H_2SO_4 as the primary aerosol in its atmosphere^{1,2}. Identification depended on comparison of the detailed shapes of the calculated and observed absorption features. The ASSESS mission provided the opportunity to observe Venus at a different phase angle, providing additional data on the structure of its upper atmosphere. The chance also arose to observe IRC+10216, which has not been studied in this spectral region with an airborne telescope.

The instrument flown consisted of a continuously variable filter wedge spectrometer plus a liquid nitrogen-cooled InSb detector system. The bandpass of the spectrometer was from 2.8 to 5.6 μ m with roughly 1.5% resolution and it was rotated continuously while taking spectra. Signal processing used a standard phase-lock amplifier system referenced to the oscillating secondary mirror of the telescope. The spectrometer and detector were mounted at the focus of the Meudon 30-cm telescope. The overall system bandpass, including the effects of the Mylar window, was approximately 3.5 to 5.0 μ m.

Unfortunately the aerodynamic loading on the Meudon telescope was so severe as to require that a Mylar window be installed over the cavity. This compromised our data in three ways: (1) Mylar has a rather broad absorption feature centred at roughly 3.4 μ m, just in the best region for studying H_2SO_4 on Venus; (2) a large offset signal due to the window was observed; and (3) condensation on the window gave some signal attenuation, though this was a minor problem in this spectral region. Nevertheless, spectra were obtained of Venus, IRC+10216, and the Moon for calibration and are being analysed.

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Airborne television observations of airglow clouds in the near infrared

AN image iscocon intensifying television system was flown to make a survey of the size, geographical distribution and frequency of occurrence of airglow clouds in the near infrared. Peterson and Kieffaber¹ have published photographs of cloud-like structures identified as OH airglow, taken at low elevation angles and with exposures of several minutes, on clear nights in New Mexico. The aim of our experiment was to establish whether airglow structures detected with the image intensifying television could be used as tracers of winds in the upper atmosphere.

The television system can detect very faint light signals (it provides at least two orders of magnitude intensification), with the option of integrating the image inside the television tube for periods from 1/25 s up to several seconds. This instrument could therefore detect and record airglow emission structures rapidly and so give good spatial and temporal resolution. Furthermore, the use of an aircraft as an observation platform makes it possible to eliminate most of the interference from meteorological clouds.

The television camera was primarily positioned looking out at 60° to the horizon, but on two flights it was situated at a 15° window. The camera had a 30° field of view. The long wavelength cut-off at the television tube was ~ 900 nm, with peak response at ~ 600 nm. A Wratten filter with short wavelength cut-off at 670 nm was placed in front of the camera lens. Peterson and Kieffaber² made observations of airglow clouds



Fig. 1 Airglow clouds looking east from an altitude of 39,000 feet on June 18 at 0930 UT (0122 LT), latitude 45°, longitude 122°W. The picture was obtained by integrating over seven television fields, that is, for 0.28 s.

at a 15° window on the same side of the aircraft throughout the mission.

We found that well defined cloud-like structures could be observed easily with the camera only from the low elevation (15°) window (see Fig. 1). The atmosphere was not sunlit below about 400 km at this position, thus excluding the possibility that these structures are noctilucent clouds.

Assuming that the aircraft speed (~ 250 m s⁻¹) is likely to be much greater than upper atmospheric wind speeds at middle latitudes, we can estimate the altitude (80–85 km) and horizontal dimension (~ 20 km) of individual bright patches of airglow.

Airglow viewed through the 60° window was diffuse, and small scale structure was not easy to identify. There were, however, variations in intensity over the television field of view, particularly when well defined structures were visible at lower elevations through the image intensifiers of the cameras of the New Mexico group. Identification of individual clouds was complicated by the aircraft roll during the longer integration times (1 or 2 s) needed at the high elevation window because of lower airglow intensities. These lower intensities indicate that the thicknesses of the airglow structures are small compared with horizontal dimensions. The general airglow brightness, seen from the 60° window, varied from day to day and from place to place; we are making a separate study of these variations from the television records.

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² Peterson, A. W., and Kieffaber, L. M., *Nature*, **257**, 649–650 (1975).

Far infrared observations of dark cold clouds and H II regions

WE present here a brief description and preliminary results of our airborne far infrared experiment (see ref. 1). At the focus of a 30-cm open-port Cassegrain telescope was a Low-type germanium bolometer, NEP of 3×10^{-14} W Hz^{-1/2}, following a four-filter set of bandwidths, respectively, 31–38 μm, 47–67 μm, 72–94 μm and 114–196 μm. Cold diaphragms from 1.57' to 6.3' were actually used². The secondary mirror was wobbling at 37 Hz, with a typical beam throw of 9', and scanning in both *x* and *y* was possible up to 30' × 30'. The stability of the system was better than 1' and was as low as 15'' during steady parts of the flights. Tracking was done by means of the star video signal (from a Nocticon tube), which was electronically processed and looped on the torque motors of the gyro-stabilisation. A PDP-11 computer was used for on-line visualisation of digitised maps.

Calibration was carried out seven times on Venus, but unfortunately not for all the flights reported on here. Significant and variable 'sky noise' (of up to 20 times the detector noise) meant that only some of the data is really usable; we therefore made systematic measurements on this 'sky noise' (to be published).

Table 1 Flux intensity in 10^{-23} W m⁻² Hz⁻¹

Source	114–196 μm	72–94 μm	46–67 μm
W51	4.4 ± 0.5	16 ± 2	42 ± 4
M17	28 ± 8	—	—
ρ Oph	14 ± 4	—	—
S131	22 ± 6	—	—

The aim of the experiment was the detection and mapping at several wavelengths of dark galactic clouds. Four sources were studied: ρ Ophiuchus, S131, W51 and M17. At this early stage of the data examination, only the last two seem to show extension and structure. Table 1 summarises the fluxes computed for these four objects assuming, for Venus, a black-body temperature³ of 220 K, and a size of 19'. We can derive for W51 an equivalent black-body temperature significantly higher than that proposed by Harvey⁴, at least 100 K. As for M17, our values agree well with those of Hoffmann⁵, assuming that the source is extended and uniformly bright. Our studies of ρ Oph and S131 confirm the high degree of correlation between CO clouds and dark regions^{6,7}.

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Operon structure of DNA transfer cistrons on the F sex factor

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The operon structure of the tra cistrons on the F sex factor has been investigated using Mu-1 bacteriophage. Twelve tra cistrons comprise one polycistronic operon 15 × 10⁶ to 20 × 10⁶ daltons long.

GENETIC analyses¹⁻⁷ of transfer-deficient (Tra⁻) mutants of the F sex factor have revealed the existence and map order of thirteen *tra* cistrons on F as summarised previously^{8,9}. Electron microscopic analysis^{10,11} of DNA heteroduplexes made with Tra⁻ mutants, has resulted in a physical map that correlates with genetic assignments as shown in Fig. 1. The physical units are kilobases (kb), an abbreviation for one thousand nucleotide bases or base pairs. Throughout this article, right and left refer to the (arbitrary) orientation used in Fig. 1. The *tra* cistrons are clustered according to function in that cistrons between 62 and 79 kb are involved in F pilus synthesis while cistrons between 80 and 94 kb have other DNA transfer-related functions but are not necessary for F pilus synthesis. We believe that *traJ*, located to the left of all other *tra* cistrons, codes for a positive control protein, necessary for the production of all other *tra* cistron gene products; thus *traJ*⁻ mutants lack all the other *tra* gene products as demonstrated by genetic tests¹².

One of the *tra* cistrons, *traS*, lies between 80 and 89 kb (between *traG* and *traD*⁷). *traS* codes for the surface exclusion phenomenon wherein F-carrying cells are poor recipients (Sfx⁺) for conjugation with other F-carrying donor cells. *traS*⁻ mutants are still transfer-proficient¹⁰. Thus the wild-type F factor exhibits a Tra⁺ and Sfx⁺ phenotype. *traJ*⁻ mutants are Tra⁻ and Sfx⁻, whereas mutants in other *tra* cistrons are Tra⁻ but Sfx⁺. A few nonsense and frameshift mutants located to the left of *traS*,

are partially surface-exclusion-deficient and exert polarity on *traS* as if it were in the same operon². None of these putative polar mutants, however, lies to the left of *traK* (68–73 kb), so they are of little use for defining the operon structure of all the *tra* cistrons. The genetic properties of Tra⁻ deletion mutants³ indicated, however, that any polycistronic *tra* operon(s) must be transcribed from left to right. Accordingly, we initiated a search for absolute polar mutants mapping in each of the *tra* cistrons, to investigate their polarity on the other *tra* cistrons and thus to define the structure of the *tra* operon(s). Mu-1 bacteriophage has been found to insert itself randomly within *Escherichia coli* DNA¹³ and such Mu-1 insertions manifest (absolute) polarity on any cistrons lying distal to the Mu-1 insertion site¹⁴⁻¹⁸ in the direction of transcription by acting as a transcriptional block¹⁹. Indeed if all the *tra* cistrons between *traA* and *traI* were in one operon, the *traA*–*traI* operon would have a length of 15 to 20 × 10⁶ daltons and thus would be the largest bacterial operon yet described.

Isolation of Mu-1 insertion mutants

The information we have on *traS* predicted that we could separate Mu insertions into two classes on the basis of whether or not they were polar on *traS*. Mu insertions polar on *traS* would lead to an Sfx⁻ phenotype, whereas Mu insertions not polar on *traS*, because they were in a different operon or operator-distal to *traS*, would be Sfx⁺. The isolation of Sfx⁻ mutants, however, is technically difficult for the following reasons. Good recipient mutants (Sfx⁻) are at a growth disadvantage in a mixed population with Sfx⁺ wild type cells because of the repeated unidirectional DNA transfer from wild type cells to Sfx⁻ mutants which results in a relative dilution (contraselection) of the

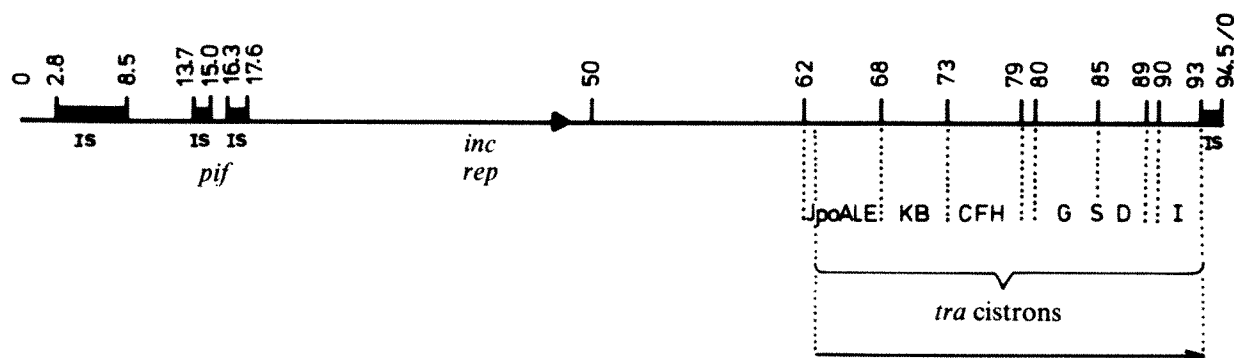


Fig. 1 The physical map of F. All units given are in kilobases (kb) according to the results of Davidson *et al.*¹¹. IS represents the known locations of insertion sequences¹¹. The location of the *pif* cistrons which code for restriction of T7 and ϕ_{11} phages is according to M. Malamy (personal communication). The location of the *rep* cistron(s) which code for vegetative replication is according to Davidson *et al.*¹¹ while the location of the *inc* cistron(s) which code for incompatibility is according to Willetts⁷. The location of the origin of DNA transfer is represented as an arrowhead and is according to Davidson *et al.*¹¹. The location of the *tra* cistrons are according to the map order defined genetically³, the correlation of genetic results between different sets of mutants by Willetts and Achtman², and the correlation of genetic results with physical results by Davidson *et al.*¹¹. The *tra* cistrons listed are known to map between the dotted lines on the basis of the length of genetically characterised deletions. *traS* lies between *traG* and *traD*⁷ but its exact location is not yet known. The location of *traL* is according to the results presented by Willetts⁶ correlated with the results presented here. The locations of the promoter (p) and operator (o) are according to the results presented here. The arrow under the *tra* cistrons depicts the transcriptional direction of the *tra* operon as demonstrated here.

Table 1 Genetic locations of Mu-1 insertion mutants

Method	No. of mutants in												Total
	J	A	E	K	B	C	F	H	G	S	D	I	
1	0	0	0	0	0	0	3	5	0	0	10	1	19
2	0	3	2	0	7	2	9	7	2	0	0	0	32
3	1	6	0	0	0	0	0	0	0	0	0	0	7

For all three methods, Mu-1 lysates were spotted on bacterial cultures of (JCFLO)⁺ derivatives of strains JC3272 (methods 2 and 3) or JC5455 (method 1)²⁴. JCFLO is a *lacI*⁻ derivative of *Flac* (F42)²⁴. After 4–6 h at 37 °C for lysogenisation, subcultures were inoculated in nutrient broth with 0.01% SDS (methods 2 and 3) or without SDS (method 1). Overnight growth at 37 °C was conducted to allow segregation and expression. For method 1 dilutions were plated and the resulting colonies screened for Tra⁻ mutants by replica-plate crosses²⁴ against a *thr::Mu-1* F⁻ recipient culture. For methods 2 and 3, the overnight cultures were diluted into nutrient broth without SDS, grown to exponential phase and mated with a Mu-1 resistant derivative of Hfr KL98. His⁺ (Str^R) recombinants were selected on minimal medium plates containing SDS. For method 2, the His⁺ recombinants were screened for Tra⁻ and Sfx⁻ mutants by replica-plate crosses²⁷ against *thr::Mu-1* derivatives of an F⁻ recipient and an (F8)⁺ donor strain, respectively. For method 3, replica-plate crosses were first used to transfer R100-1 from a Mu-1 resistant (R100-1)⁺ donor culture to the His⁺ recombinants and these (R100-1) derivatives were then screened for M12-resistant clones. All the mutants listed were isolated from independent cultures and are of independent origin. A further 33 mutants isolated by method 2 are described in the text.

Sfx⁻ mutants. By incorporating 0.01% sodium dodecyl sulphate (SDS) in liquid and solid media, mating pair formation can be prevented, thus facilitating the isolation of Sfx⁻ mutants²⁰. The procedure used (ref. 20) consisted of including SDS in all growth media to prevent loss of Sfx⁻ mutants and included an enrichment for Sfx⁻ mutants by selecting recombinants after a mating between the mutagenised population and an Hfr donor. The recombinants were enriched approximately 100-fold for Sfx⁻ mutants compared with the (poor recipient) Sfx⁺ wild type.

The above information enabled us to design three mutant isolation procedures intended to yield (Mu-1)⁺ mutants which were, respectively, Tra⁻ Sfx⁺, Tra⁻ Sfx⁻ and *traJ*⁻. In the first procedure, we screened 39,000 (Mu-1)⁺ lysogens of an (*Flac*)⁺ strain for Tra⁻ mutants after an overnight growth step in the absence of SDS. As expected, a number of Sfx⁺ mutants (11/19) was isolated, in addition to eight Sfx⁻ mutants which escaped the natural selection against such mutants during growth of mixed populations. For the second procedure, SDS was present in all growth media to prevent loss of Sfx⁻ mutants and these mutants were then enriched by a mating with an Hfr donor culture followed by selection of recombinants. SDS was omitted during the mating. We screened 13,000 recombinants of (Mu-1)⁺ lysogens of an (*Flac*)⁺ strain and 65 Tra⁻ Sfx⁻ mutants were isolated. The first 32 analysed genetically by complementation tests seemed to represent an adequate sample of the *tra* cistrons except *traJ*, and the remaining mutants were tested only to ensure that none carried a *traJ*⁻ mutation. The complementation tests of the 65 mutants just described demonstrated that each mutant gave poor complementation for one or more clustered cistrons. We assigned a tentative genetic location for each Mu-1 insertion as being in or immediately to the left of the left-most cistron which was poorly complemented (Fig. 1). This assignment is demanded by the results of complementation tests with deletion mutants³ which demonstrated that any

polycistronic operons are transcribed from left to right. The genetic locations of the mutants are presented in Table 1. No *traJ*⁻ mutant was isolated by procedures 1 or 2 and the complementation results (discussed in detail below) did not enable any conclusion to be drawn about whether or not *traJ* was in the same operon.

The third procedure took advantage of the special properties of *traJ* and *traA*. As described above, *traJ*⁻ mutants lack the gene products of the other *tra* cistrons including *traA*^{12,21}. Willetts²¹ has shown that cells carrying either a *traJ*⁻ mutant or a *traA*⁻ mutant of F, plus the sex factor R100-1 (which has considerable DNA homology with F within the *tra* region²²) are relatively resistant to M12 male-specific bacteriophage, while cells carrying R100-1 and an F factor mutant in any other *tra* cistron are sensitive. Although the mechanism of this phenomenon is still not totally clear, we took advantage of it as a screening procedure for *traJ*⁻ mutants. We thus repeated the procedures used in the second method but in addition a strain carrying the R100-1 factor was mated with the selected recombinants; (R100-1)⁺ progeny were screened for M12-resistant clones by replica plating methods. The corresponding non-R100-1 containing colonies on the master plates were purified and analysed. Seven mutants were isolated out of 200,000 recombinants of which six carried Mu-1 in *traA* and one carried Mu-1 in *traJ*.

All the mutants listed in Table 1 have been shown to carry Mu-1 on the F factor since after acridine orange curing²³ to remove the F factor, the ability to produce Mu-1 phage was concurrently lost. All 58 mutants in Table 1 were tested for their DNA transfer ability and their surface-exclusion ability by replica-plating methods and in 33 out of 58 cases, chosen as representative of the various *tra* cistrons, by quantitative measurements as well. The qualitative and quantitative results (Table 2) were essentially the same as those described for deletion mutants^{3,7} which contained sequentially longer deletions

Table 2 Properties of Mu-1 insertion mutants

Mu location	No. of mutants	TE*	SEI†	F ₁	F ₂	QB ³
<i>traI</i>	1	≤ 3 × 10 ⁻⁷	67	S	S	S
<i>traD</i>	3	≤ 1 × 10 ⁻⁵	≥ 200	S	R	S
<i>traD</i>	1	≤ 1 × 10 ⁻⁵	46	S	R	S
All other cistrons	27	≤ 1 × 10 ⁻⁶	1–3	R	R	R
<i>traH</i>	1	≤ 1 × 10 ⁻⁶	13	R	R	R

S, Sensitive; R, resistant.

*The transfer efficiency²⁴ (TE) is defined as the number of (*Flac*)⁺ exconjugants derived from the F⁻ parent which are formed in a mating between the mutant strain used as donor and a suitable F⁻ recipient. The value is normalised for the number of mutant cells added to the mating mixture.

†The surface exclusion index²⁴ (SEI) is obtained by dividing the number of recombinant colonies measured in a mating between an Hfr and an F⁻ culture by the number measured in a parallel mating between the Hfr and a test culture. A value of 0.5 to 1.0 for the TE and 100 to 200 for SEI are typical of a wild type (*Flac*)⁺ culture.

Table 3 Conjugational complementation tests with reference mutants

Location of mutation	Sex factor	Donor mutation								
		<i>traJ90</i>	<i>traA1</i>	<i>traE18</i>	<i>traB2</i>	<i>traC5</i>	<i>traF13</i>	<i>traH80</i>	<i>traG24</i>	<i>traD14</i>
J	MPFL10	< 0.008	0.8	0.6	1.3	0.6	1.6	0.4	0.5	1.2
A	MPFL6	0.75	0.01	0.01	0.008	0.03	0.2	0.03	0.01	0.2
E	MPFL5	1.9	0.6	0.07	0.04	0.02	0.4	0.3	0.3	0.4
B	MPFL4	1.2	0.4	0.9	0.03	0.04	0.3	0.1	0.09	0.4
C	MPFL11	1.2	0.6	0.9	1.0	0.03	0.04	0.1	0.03	0.07
F	JCFL171	1.0	1.2	1.3	2.3	1.4	0.01	0.02	0.05	0.01
H	JCFL161	2.8	1.9	1.9	3.1	2.6	1.3	0.02	0.1	0.05
G	JCFL184	0.7	1.9	1.6	3.0	1.6	2.6	1.9	0.005	0.09
D	MPFL2	0.8	1.2	2.0	1.0	1.1	0.3	0.7	0.9	0.03
Right of D	JCFL146	1.1	2.5	3.2	2.5	2.8	0.8	1.6	3.7	1.4

These results were obtained with complementation tests performed 144 at a time in Microtiter plates as before^{1,23} but with an improved normalisation method. The values representing poor complementation are underlined. Each test consists of two successive crosses in which first an amber-suppressible *tra* mutant was introduced from a Su^+ host into the Su^- (Mu-1)⁺ insertion mutant strains. The degree of complementation was measured in the second cross by measuring the transfer efficiency of this population to an F^- (Mu-1)⁺ recipient after first killing the Su^+ primary donor with T6 phage. Normalisation was possible by always including an *Flac tra^+ lacZ^-* mutant (amber *lac* mutation) as donor and a different *Flac tra^+ lacZ^-* mutant (temperature-sensitive *lac* mutation) as recipient in every set of crosses. When F transfer by complementation due to the *tra^+ lacZ^-* donor was tested, a $Su^- F^-$ host was used in the second cross so that transfer of only the complemented mutant was measured. When F transfer by complementation due to the *tra^+ lacZ^-* recipient was tested, a $Su^+ F^-$ host was used in the second cross (transfer of the *Flac lac^-* mutant itself is not seen since these cells are *Lac^-* at the incubation temperature of 37 °C). The use of these two *Flac lacZ^-* mutants enabled us first to normalise the various donor cultures against the *lacZ^-* recipient mutant where all are complemented, and second to normalise the various recipient cultures against the *lacZ^-* donor mutant which also complements all of them. These two normalisations remove any effects due to different recipient ability of the individual recipient cultures and to different donor ability of the individual donor cultures.

running from *traL* leftwards. All mutants mapping to the right of *traS* were Sfx^+ and Tra^- and made F pili. All mutants mapping to the left of *traS* were Sfx^- and Tra^- and male specific phage resistant. The mutants tested only by replica-plate methods yielded qualitatively identical results.

The possibility existed that Mu-1 was not integrated at the site of the *tra* mutation, but elsewhere on *Flac* in the various mutants. This possibility was tested for a representative group of the mutants by using transduction with P1 phage to introduce *tra^+* DNA from a wild-type *Flac* element, followed by a mating to select Tra^+ recombinants. The mating was performed at 33 °C with a lysogenic F^- recipient with a temperature-sensitive Mu-1_{ts62} prophage integrated in *thr*. Since the recipient is immune to Mu-1 phage at 33 °C, both *Flac* elements either carrying Mu-1 or not carrying Mu-1 will establish themselves after transfer, and zygotic induction will not occur. At 42 °C the immunity due to the chromosomal Mu-1 prophage disappears and the cells die due to temperature induction unless the *Flac* element carries a Mu-1 prophage. Thus we could determine how often Tra^+ recombinants that were still lysogenic for Mu-1 were formed. Fifty colonies were tested from each cross: in all cases the Tra^+ colonies did not contain Mu-1 on *Flac* and the corresponding mutants must therefore carry Mu-1 inserted at the site of the *tra* mutation.

Operon structure

Table 3 presents results of complementation tests, using the conjugational complementation method¹ with the same

representative mutants mentioned above. Each Mu-1 insertion is polar on the neighbouring cistrons to the right regardless of where Mu-1 is inserted, except for Mu-1 in *traJ*. Furthermore, most of the Mu-1 insertions showed clear polarity on all the cistrons to the right of the insertion, although in many cases the polarity was not absolute. The Mu-1 insertion in *traJ* showed no polarity effects. The polarity pattern indicates first, that the direction of transcription for *traA* to *traD* is from left to right (Fig. 1) in agreement with results from deletion mutants³. Second, the 11 cistrons *traA* to *traD* must lie in one operon. These results also agree with the properties of three polar *traK* mutants, one polar *traC*, and one polar *traH* mutant which showed polarity through to *traS*^{1,2,20}. In addition 15 other amber, ochre, UGA or frameshift mutations, lying between *traB* and *traS* have also been shown to exert some polarity on *traS* (unpublished observations of M. A. and N. Willetts).

The results in Table 3 also indicate some residual complementation in tests between Mu-1 insertion mutants and mutations located distally in the *tra* operon. Such complementation was not observed in tests between deletion mutants and the same point mutations³ and therefore this low level of complementation demonstrates that the Mu-1 insertions have not deleted the cistrons being tested. This residual complementation is discussed further below.

The cistrons *traL*, *traK* and *traI* are not testable by the method described above for technical reasons, and so these three cistrons were tested by the P1 transductional method² (Table 4). These results taken together with those of

Table 4 P1 transductional complementation analysis with reference mutants

Location of Mu-1	P1kc lysate from	% complementation with recipient mutation		
		<i>traL311</i>	<i>traK105</i>	<i>traI65</i>
<i>traJ</i>	MPFL10	35	10	16
<i>traA</i>	MPFL6	0.05	< 0.01	< 0.1
<i>traE</i>	MPFL5	86	< 0.01	< 0.1
<i>traB</i>	MPFL4	101	9	< 0.1
<i>traC</i>	MPFL11	112	96	< 0.1
<i>traF</i>	JCFL171	78	73	< 0.1
<i>traH</i>	JCFL161	167	6	< 0.1
<i>traG</i>	JCFL184	118	11	< 0.1
<i>traD</i>	MPFL2	199	257	< 0.1
<i>traI</i>	JCFL146	179	272	0.3

These tests were performed as described previously² except that P1kc was used instead of P1virA. Negative values are underlined. P1kc lysates grown on the (Mu-1)⁺ insertion mutants were used to transduce strains carrying an *Flac* mutant carrying either *traL311*, *traK105* or *traI65* mutations. Complementation of the resulting abortive transductants was then assayed by crosses with an F^- strain. All results were normalised to a value of 100% for a P1kc lysate grown on a wild type *Flac* element after previous normalisation for the few colonies obtained due to leakiness in control transductions with a P1kc lysate grown on an F^- strain.

Willetts define the map location of *traL* as being between *traA* and *traE*. Furthermore Mu-1 insertions between *traA* and *traD* exert polarity on *traL*, *traK* and *traI* only when they lie to the left of that cistron. The Mu-1 insertion in *traI* did not manifest any polar effects.

The conclusion that the *tra* operon extends to *traI* was reinforced by the results of a third method. Willetts²¹ has shown that *traI*⁻ and *traJ*⁻ point mutants are not complemented for DNA transfer by the related sex factor R100-1 although point mutants in all other *tra* cistrons are complemented. Tests of (R100-1)⁺ derivatives of point mutants and of Mu-1 insertions in *traJ*, *traA*, *traC*, *traD* and *traI* confirmed these conclusions for the point mutants but indicated that all the Mu-1 insertions were not complemented by R100-1. These results are those expected if Mu-1 insertions between *traA* and *traD* exert polarity on *traI*. Thus all the cistrons between *traA* and *traI*, inclusive, form one operon transcribed in the direction *traA* to *traI* and henceforth referred to as the *tra* operon. *TraJ* must be part of a second operon, whose transcriptional direction is unknown.

The residual complementation shown in Table 3 in crosses where polarity was present might be due to reinitiation within the *tra* operon, to Mu-1 insertions not being absolutely polar, or to the formation of *Tra*⁺ recombinants. The complementation seen is, however, at the limit of resolution of the method used for Table 3. The nature of the complementation was therefore investigated by a much

traH, *traG*, *traS*, *traD*, and *traI* lie in one polycistronic operon. These conclusions are strengthened by the properties of other polar mutants available which were isolated after nitrosoguanidine or ethyl methanesulphonate treatment^{1,2,20,24}. Figure 1 shows that this operon has a maximal length of 93–62=31 kb. This stretch, however, includes *traJ* which is in a second operon. Since *traI* is to the right of 90 kb and *traE* is to the left of 68 kb, a minimal estimate is provided by assuming 0.3 kb for *traI* and 1 kb for *p o traA traL traE* yielding 90–68+1.3=23 kb. Thus the operon is 23–31 kb (or 15×10⁶–20×10⁶ daltons) long. If the mRNA is synthesised as one continuous piece it would be approximately 7×10⁶–10×10⁶ daltons long and take 7–10 min to transcribe at the rate of 55 nucleotide base pairs per second²⁵.

traA through to *traH* code for the synthesis of F pili and *traA* has been found to code for the F pilus subunit (personal communication from C. C. Brinton, Jr). *traG*, *traD* and *traI* mutants synthesise F pili and even form mating pairs with F⁻ cells²⁴. No DNA transfer ensues, however, from these mating pairs and *traG*, *traD* and *traI* probably code for enzymes involved directly in DNA transfer. *traS* codes for surface exclusion and expression of *traS* prevents DNA transfer from *Tra*⁺ cells²⁰. Thus, although these cistrons code for different functions, they are all DNA transfer-related.

Surface exclusion has a strong evolutionary advantage since the *Tra*⁺ *Sfx*⁻ mutants that we have isolated are

Table 5 Conjugational complementation tests in Rec⁺ and Rec⁻ hosts

Recipient <i>Flac</i> sex factor	Location of Mu-1	<i>Fhis</i> donor							
		Host	<i>Tra</i> ⁺	<i>traJ90</i>	<i>traA1</i>	<i>traE18</i>	<i>traB18</i>	<i>traF13</i>	<i>traD83</i>
MPFL6	A	Rec ⁺	0.94	0.37	≤ 0.0004	≤ 0.001	≤ 0.0003	0.005	0.03
MPFL6	A	Rec ⁻	0.79	0.39	≤ 0.0003	0.0009	0.0009	0.0007	0.005
MPFL5	E	Rec ⁺	0.20	0.16	0.05	0.005	0.05	0.004	0.07
MPFL5	E	Rec ⁻	0.90	0.68	0.14	0.007	0.0003	0.002	0.01
JCFL184	G	Rec ⁺	—	1.4	0.68	0.20	0.60	0.62	0.005
JCFL184	G	Rec ⁻	—	0.96	1.51	1.61	3.22	0.36	0.004

Tube complementation tests¹ were performed using transient heterozygote cells carrying both *Fhis tra*⁻ and *Flac tra*::Mu-1 mutants. The transfer efficiency per heterozygote RecA⁺ or RecA⁻ cell is shown. The Rec⁺ hosts were the same strains used in Table 2 whereas the Rec⁻ hosts were closely related *recA58* derivatives.

more sensitive tube complementation method¹. For these tests the *Flac* Mu-1 insertion mutants were transferred to a RecA⁻ host. Furthermore, *Fhis tra*⁻ mutants were isolated after recombination between *Fhis* (F57¹) and the appropriate *Flac tra* mutants containing point mutations. The genetic character of both the new *Fhis tra* mutants and the transferred *Flac* Mu-1 insertion mutants were confirmed with the same methods as used for Table 3.

Fhis/Flac heterozygote cells can be assayed by the formation of Lac⁻/Lac⁺ sector colonies and thus it is possible to estimate directly the complementation efficiencies per heterozygote cell. The results of such tests are presented in Table 5. Other tests between *Flac* mutants with Mu-1 in *traF*, *traH*, *traB* and *traD* and mutants carrying *Fhis traA1* or *traD83* were also performed with comparable results (data not shown). The conclusion from all these tests is that some residual complementation is in fact seen in both RecA⁺ and RecA⁻ hosts in crosses where polarity should result in no complementation. Complementation was accompanied by recombination in the Rec⁺ host but no *Tra*⁺ recombinants were formed in any of the crosses in the RecA⁻ host (data not shown). Thus either Mu-1 insertions are not absolutely polar in this system or there is some low level reinitiation within the *tra* operon.

Implications

The data presented here on Mu-1 insertion mutants demonstrate that *traA*, *traI*, *traF*, *traK*, *traB*, *traC*, *traE*

unstable in normal growth conditions²⁰. This instability would result in the selection of *Tra*⁺ *Sfx*⁺ mutants from a primaevial *Tra*⁺ *Sfx*⁻ population in nature in conditions where transfer was possible. It has possibly also been this selection pressure which resulted in the incorporation of *traS* into the *tra* operon since surface exclusion is thus expressed whenever the potential for DNA transfer is expressed.

The elucidation of the *tra* operon structure also simplifies any models to explain the mechanism of action of the *traI* gene product. *traI* is a positive control gene^{1,2} and *traI*⁻ mutants lack the gene products of all the other *tra* cistrons¹². A simple model to explain these relationships is that the *traI* gene product is necessary for transcription of the *tra* operon. In the case of the F factor, *traI* is always expressed as is the *tra* operon. Most R factors are repressed²⁶ and in at least the case of R100 the mechanism seems to be by the prevention of the synthesis of the *traI* gene product^{12,27,28}. Thus R factors can provide economy and repress the formation of sex pili, DNA transfer enzymes and surface exclusion by indirectly repressing the *tra* operon in a cascade fashion. There are always a few non-repressed cells in a repressed population which can transfer R factor DNA immediately on contact with R⁻ cells. After transfer to the R⁻ cells the *traI* gene product is synthesised before the repressor²⁸, the *tra* operon is turned on and the new R⁺ cell can itself transfer R factor DNA to any other R⁻ cells. Thus, essentially, the R factor DNA

transfer occurs when a repressed R^+ population is mixed with an R^- population. As soon as all the cells have received the R factor, surface exclusion prevents further DNA transfer. In the absence of DNA transfer no new *traJ* gene product is synthesised due to the repressor and dilution of the *traJ* gene product eventually leads to a newly repressed population in which neither *traJ* nor the *tra* operon are expressed.

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letters to nature

Discovery of powerful transient X-ray source A0620–00 with Ariel V Sky Survey Experiment

ON August 3 a faint new X-ray source was discovered (at 15 Ariel counts s^{-1}) near the boundary of the Monoceros and Orion constellations and 6° from the galactic plane, during routine monitoring of the Milky Way with the Ariel V Sky Survey Experiment (SSE). During the following week, the source—designated A0620–00—increased

rapidly in intensity to become the brightest in the sky (at 2–18 keV). The X-ray light curve from continued observation with the SSE to August 16 is shown in Fig. 1. The ordinate is in Ariel counts s^{-1} and for comparison, the corresponding count rates for the Crab Nebula and Sco X-1 are 400 s^{-1} and 3,200 s^{-1} , respectively. The precursor peak on August 6 is real and is qualitatively similar to the feature seen¹ in the rise to maximum intensity of the earlier Ariel transient A1524–62. Subsequent observations of A0620–00 with the Ariel V experiments pointing along the spin axis of the spacecraft have shown the intensity to have remained very high at least until August 27.

Figure 2 shows a copy of the Palomar Observatory Sky Survey chart marked up with the initial SSE X-ray source position, an improved Ariel V location² and the optical counterpart³ discussed in the following letter⁴. Figure 3 compares an expanded blue print of the region from the Palomar survey and a post-transient blue photograph taken with the UK Schmidt telescope at Siding Spring. In spite of the considerably reduced exposure of the new plate, limited by sky brightness—since A0620–00 is at present visible only near twilight—the considerable brightening of the optical star ($\Delta M_B \sim 8$) is readily apparent. Detection of a short lived radio source coincident with the optical star is reported in a later letter⁵.

Most current theories of optical novae^{6,7,8} invoke unstable mass transfer in a close binary system in which the accreting star is probably a white dwarf. The transient nature of A0620–00 indicates that this may have a similar origin, but the high X-ray luminosity strongly suggests a qualitative difference from optical novae (none of which have been detected by, admittedly incomplete, X-ray surveys), probably in that for an 'X-ray nova' to occur the accreting object has to be a neutron star or black hole. In the present case it is of particular importance to determine the distance and thus the X-ray luminosity of A0620–00. If, as seems likely, the source reached a maximum intensity from August 12 corresponding to an accretion rate at the Eddington limit, then the measured X-ray flux above 1 keV ($\sim 650 \text{ keV cm}^{-2} \text{ s}^{-1}$) corresponds to a limiting X-ray luminosity ($1.3 \times 10^{38} \text{ erg s}^{-1}$) for a secondary of mass M_\odot at a distance of 1 kpc. A preliminary analysis of high dispersion spectra of the optical star (Dr T. Gull, personal communication), reported to show marked interstellar absorption features⁹, gives a minimum distance of 600 pc,

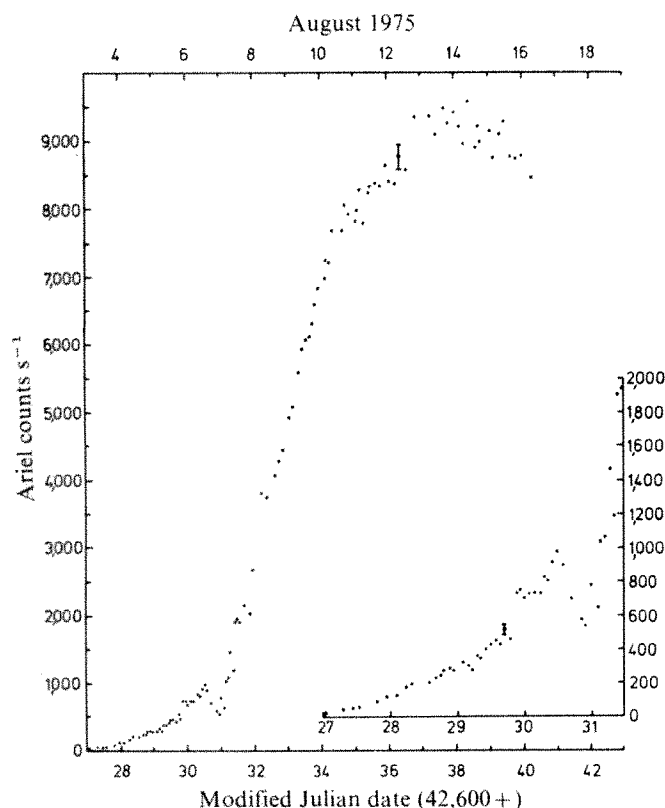


Fig. 1 The X-ray light curve of A0620–00 obtained with the SSE at 2–18 keV. The error bars are $\pm 1 \sigma$ statistical errors at the lower intensity end (see inset); towards the peak the major error arises from uncertainties of 0.1° in the spacecraft attitude.

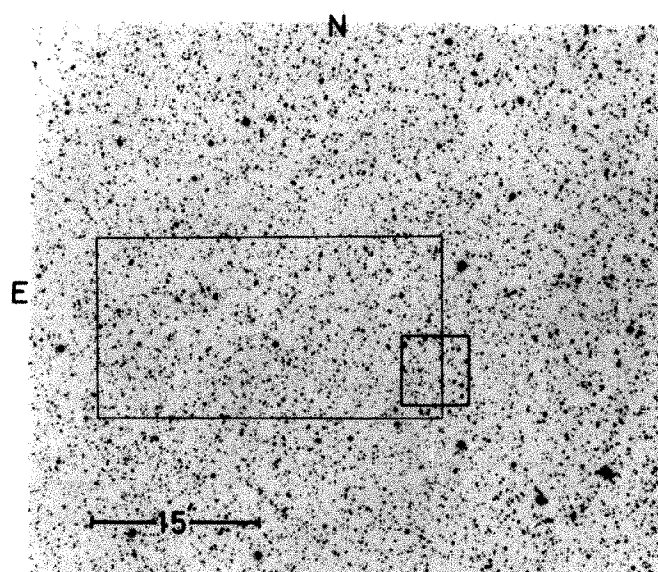


Fig. 2 Palomar Observatory Sky Survey red photograph showing the initial and improved Ariel V X-ray error boxes (90% confidence level) and the subsequently identified optical counterpart (copyright: National Geographic Society, Palomar Observatory Sky Survey).

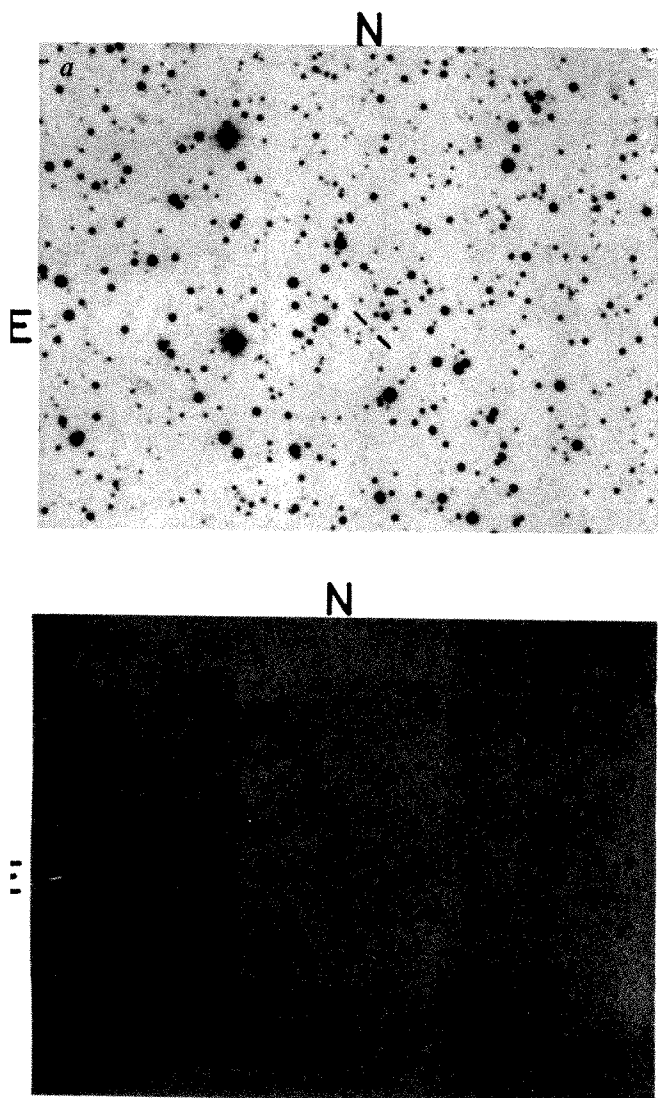


Fig. 3 A comparison of the optical counterpart on the blue POSS photograph with a post-transient blue plate taken with the UK Schmidt telescope at Siding Spring (courtesy: Science Research Council, UKSTU)

whereas the discovery of an earlier optical outburst of A0620-00 in 1917 (ref. 10) has been interpreted in terms of a recurrent nova which, if of typical luminosity for a 60-yr period nova, would indicate a distance of 5,700 pc. If confirmed, a distance of this order would imply that the accreting component of A0620-00 is a massive black hole.

Finally, a search for periodic variation in the X-ray intensity of A0620-00 has shown no modulation ($>3\%$) over time scales of 200 s – 2 d.

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The X-ray spectrum of A0620-00

A REMARKABLE change in the X-ray spectrum of the transient source A0620-00 (ref. 1) was observed by the Ariel V Sky Survey Experiment (SSE) during the rise to maximum brightness. The SSE detector being used has an effective area (behind its field collimator) of 290 cm² and covers the energy band 2–18 keV in 8 channels. The channel boundaries are marked in Fig. 1, which shows sample pulse height spectra taken as the source intensified. These raw data have not been corrected for the detector efficiency and at this stage include no input spectral assumptions. Two points emerge clearly: when first detected the X-ray spectrum of A0620-00 was hard and remained so as the overall intensity rose to the precursor peak and, secondly, the subsequent rise to maximum intensity was entirely due to a strong enhancement of X rays below 10 keV, giving a marked softening in the overall spectrum. In fact, the observed flux above 10 keV actually fell during this period.

To quantify these changes, the pulse height spectra have been compared with a range of input spectra defined by a spectral parameter (power law index or thermal spectrum kT) and cutoff energy, folded with the known detector response and normalised to the observed data. Table 1 summarises the best fits obtained for spectra at different times in the X-ray light curve, χ^2 indicating the quality of a particular fit. The notable softening of the spectrum as the source brightens can now be quantified as, for example, an increase in the power law index from 0.6–0.7 in the precursor peak to ~ 4.0 near maximum brightness. The corresponding thermal spectral fits show a corresponding decrease in kT from ~ 30 to 1.5 keV. The rather large values of χ^2 indicate that the spectrum is generally too complex to be fully described by a single parameter fit, most spectra having excess observed counts in the upper one or two energy channels.

Figure 2 shows the input power law spectrum which matches the data quite well from 2 to 15 keV for modified Julian date (MJD) 40.5, just after maximum brightness. Also shown are two higher energy points obtained over the period MJD 44.5–47.5 with the Imperial College scintillation counter on Ariel V (A. Engel, personal communication), during which time the intensity remained steady. Again, the highest energy SSE spectral point shows some excess counts, and the presence of a harder component above 15 keV is supported by the Imperial College data. The best fit power law of Fig. 2 includes

Table 1 Parameters of best-fit X-ray spectra

Modified Julian date(42,600+)	Power law input spectrum				Thermal input spectrum			
	$I(E) = AE^{-\alpha} \exp(-E_a/E)^{8/3} (\text{keV cm}^{-2} \text{s}^{-1} \text{keV}^{-1})$				$I(E) = \frac{A \exp(-E/kT)}{E^{0.3}} \exp(-E_a/E)^{8/3} (\text{keV cm}^{-2} \text{s}^{-1} \text{keV}^{-1})$			
	$\alpha(\pm 2\sigma)$	$E_a \pm 2\sigma$ (or 2σ upper limit)	A	χ^2_{min}	$kT(\text{keV})$	$E_a(\text{keV})$	A	χ^2_{min}
28.8–29.4	0.65 ± 0.15	< 1.0	5.5 ± 0.6	56	20^{+15}_{-5}	< 0.7	3.7 ± 0.4	83
30.1–30.5	0.6 ± 0.15	< 1.3	13.2 ± 2.8	27	29^{+40}_{-15}	< 1.0	9.9 ± 1.6	38
31.0–31.4								
(a) $E < 6.2 \text{ keV}$	1.4 ± 0.1	< 1.0	71	12	8.7	< 0.5	22	280
(b) $E > 6.2 \text{ keV}$	0.2 ± 3.0							
32.72	3.1 ± 0.2	< 1.2	$(1.83 \pm 0.47)10^3$	31	1.36 ± 0.1	< 0.75	950 ± 110	152
33.14	3.7 ± 0.25	1.85 ± 0.25	$(7.1 \pm 2.3)10^3$	19	1.35 ± 0.07	< 0.8	$(1.18 \pm 0.17)10^3$	78
34.20	3.75 ± 0.15	1.9 ± 0.15	$(11.9 \pm 3.3)10^3$	27	1.37 ± 0.07	< 0.6	$(1.4 \pm 0.2)10^3$	100
35.10	4.0 ± 0.2	2.15 ± 0.15	$(8.8 \pm 2.6)10^3$	25	1.36 ± 0.05	< 0.75	$(6.1 \pm 0.5)10^3$	74
40.5	4.15 ± 0.2	2.6 ± 0.2	$(20 \pm 7)10^3$	22	1.52 ± 0.1	$1.1^{+0.4}_{-1.1}$	$(1.05 \pm 0.3)10^3$	26

a finite low energy cutoff of $E_a = 2.6 \text{ keV}$ and examination of all the existing spectra suggests a significant low energy attenuation becoming evident as the source intensifies.

The spectral evolution reported above provides interesting insight into the nature of A0620–00. Considering a binary model with unstable mass transfer, as referred to briefly in the preceding letter, the hard ‘precursor’ emission may be interpreted as arising from the hottest regions of an accretion disk lying close to the compact star. Then, as further mass is accreted, the disk grows rapidly, giving a corresponding increase in the overall X-ray intensity. Since the outer parts of this enlarged disk will be cooler the resulting X-ray spectrum will soften. Furthermore, the reduction in flux above 10 keV as the source increases towards maximum brightness may well be due to the hot inner regions of the disk being partially

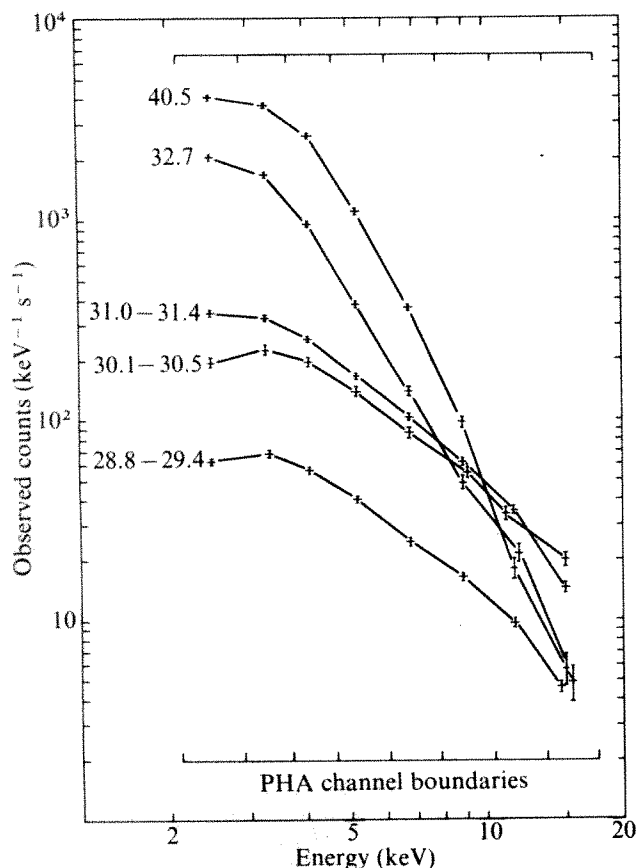


Fig. 1 Pulse height spectra of A0620–00 obtained during the rise to maximum brightness. The time of each spectrum is marked in MJD and error bars are $\pm 1\sigma$ for each spectral channel.

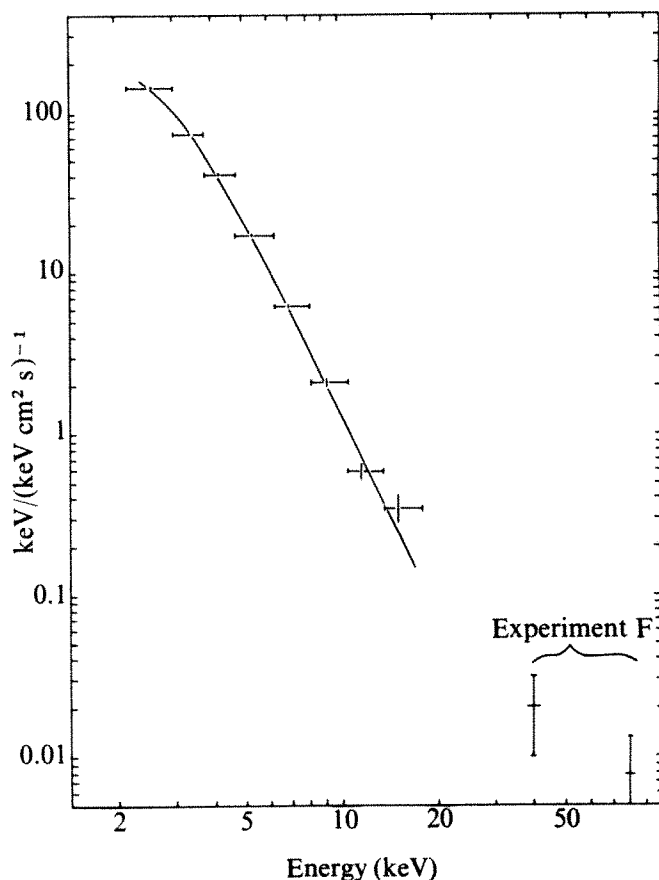


Fig. 2 The best-fit power law spectrum for the SSE data of MJD 40.5 (spectral index 4.1, low energy cutoff 2.6 keV) together with higher energy points from the Imperial College crystal spectrometer (experiment F on Ariel V) taken during MJD 44.5–47.5. Error bars are $\pm 1\sigma$.

obscured by the mass of outlying gas. A detailed analysis of the light curve and spectral evolution of A0620–00 presented here may be anticipated to yield important details on the accretion flow and disk formation in such close binary systems.

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Observations of A0620-00 at 962 and 151 MHz

FOLLOWING the discovery by the X-ray astronomy group at Leicester University of a large increase in the X-ray flux of A0620-00, observations at 962 MHz and 151 MHz have been made at Jodrell Bank. During the period August 16-29, observations were made at 962 MHz with the MK II-MK III long baseline radio-link interferometer. This instrument has a spacing of 23.7 km giving a lobe size of $\sim 3''$ at this frequency. The radio-link is phase compensated so that integrations can be made for several hours.

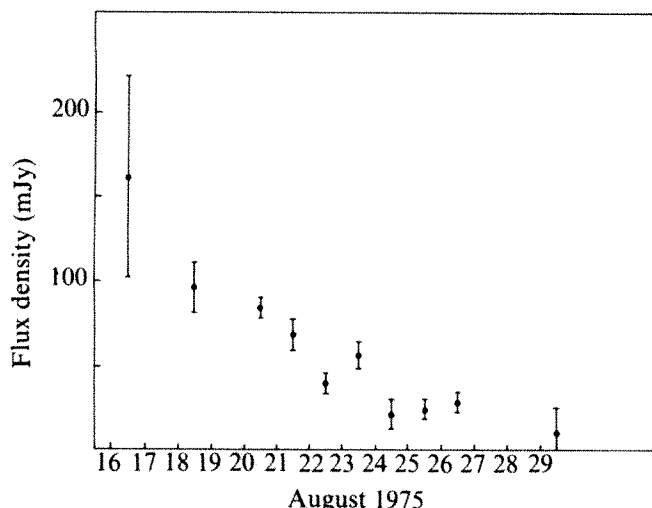


Fig. 1 Flux density of A0620-00 at 962 MHz with a resolution of $3''$

On August 16 and 18, the instrument was used to survey an area 0.5° by 0.5° centred on the position of the X-ray source¹. For a source at declination 0° the synthesised beam after integrating from hour angle 1.5 – 3.5 h is $5''$ in right ascension and $30'$ in declination. In the area surveyed only one source, at the extreme edge of the field, was significantly above the noise level. On August 19 the position of an optically variable object, possibly associated with the X-ray source, was communicated to us². The right ascension of this object and the observed radio source agreed to within $6''$ making an association likely. Further observations were therefore restricted to a smaller area of sky surrounding the position of the radio source and optical object. Thus a wider bandwidth could be used, giving an increased sensitivity and enabling the position to be measured with greater accuracy. With the 4-MHz bandwidth the r.m.s. noise on an amplitude measurement after a 2-h integration was 6 mJy. The position measured on August 18 was: $\alpha = 06$ h 20 min 11.4 ± 0.27 s; $\delta = -0^\circ 17 \pm 7'$ (1950.0). Subsequently, more accurate measurements of the X-ray source position confirmed the radio identification^{3,4}. No significant variation of fringe amplitude with hour angle was detected during the observations, thus indicating that the angular size of the radio source was less than $3''$. Measurements of the flux density of the radio source were continued until August 29 and showed a decreasing intensity with time as shown in Fig. 1.

During August 12-15 observations were also made at 962 MHz using the MK II radio telescope. Total power measurements indicated that the flux density of the source was less than 5 Jy. On August 17, 20, 23 and 24 observations were made using the MK IA-Defford radio-link interferometer at 151 MHz. This instrument has a baseline of 127 km which gives a lobe size of $3''$ at this frequency. No radio emission was observed down to a sensitivity limit of 250 mJy. At this frequency, however, interstellar scattering may increase the apparent angular

size of a source near the galactic plane, as in the case of Cyg X-3 (ref. 5), and this could explain why no source was observed in the present observations at 151 MHz.

If the decrease of flux density shown in Fig. 1 is expressed as an exponential decay, the time for the flux density to fall by a factor of $1/e$ is 5.2 d. The data may also be fitted to a power law of the form $t^{-\beta \pm 1}$ on the assumption that the outburst originated on August 3.0 UT. On the simple van der Laan model⁶ of uniform expansion of an optically thin cloud of relativistic electrons, this would correspond to a radio spectral index, α , of -0.25 ± 0.25 where α is defined by $S = S_0 \nu^\alpha$.

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The optical counterpart of A0620-00 before its eruption

AN accurate position of the optical counterpart of A0620-00 discovered by Boley and Wolfson¹ has made possible the identification of the image of the star on the Palomar Observatory Sky Survey (POSS) charts.

The position was measured on two 10-min exposures on Eastman Kodak Ila0 plates taken on the 13-inch astrographic telescope at Herstmonceux on August 27-28 and 28-29, 1975 relative to standard positions of AGK3 stars, using a Zeiss x - y measuring engine. It was reduced using the methods described by Murray, Tucker and Clements². The mean position of the Boley-Wolfson star which appears at $B \sim 12.2$ on both plates is: $\alpha = 06$ h 20 min 11.176 ± 0.021 s; $\delta = -00^\circ 19' 10.80 \pm 0.32''$ (1950.0), where r.m.s. errors are quoted. There is a substantial contribution to these errors from the uncertainty of the system defined by the AGK3 stars and from the possible effects of refraction if the identification has a different colour from the AGK3 stars. The right ascension lies about 3 s.d. ($1.20 \pm 0.35''$) from that of the radio object measured at the Mullard Radio Astronomy Observatory³. The position is, however, within the X-ray error boxes measured by the Leicester⁴ and Birmingham-Mullard Space Science Laboratory⁵ experiments on Ariel V and by the Massachusetts Institute of Technology experiment⁶ on SAS-3.

The positions of a number of stars on the POSS charts (from originals taken on November 18-19 1955) were also determined with respect to the same AGK3 standards from measurements made with a Faul Coradi Coradigraph x - y machine. Similar reductions showed that one star, identified in Fig. 1, lies at: $\alpha = 06$ h 20 min 11.202 ± 0.023 s; $\delta = -00^\circ 19' 10.45 \pm 0.35''$ (1950.0).

In comparing these two positions the errors of the positional system defined by the AGK3 stars cancel and so the difference in position has smaller errors than the appropriate combination of the errors in the positions. The differences between the measurements obtained on the POSS and the 13-inch plates are: $\Delta\alpha = +0.39 \pm 0.38''$ and $\Delta\delta = +0.35 \pm 0.38''$. Since the positions agree very well and the next nearest object on the POSS is $\sim 15''$ away, the star identified in Fig. 1 is very likely to be the optical counterpart before its eruption.

Eye estimates on the POSS give magnitudes in 1955 for the counterpart of $B \sim 20.5$, $B - R \sim 3.6$ where the errors are likely

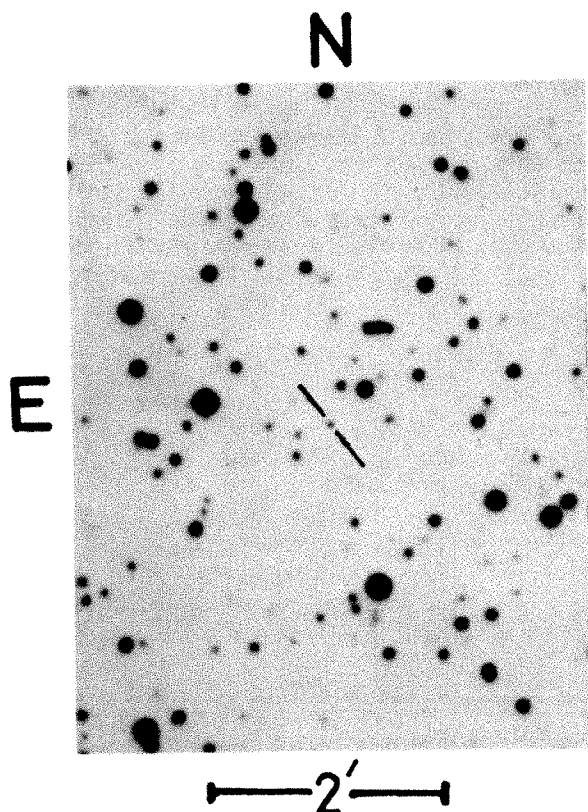


Fig. 1 Chart identifying the image of the optical counterpart of A0620-00, before its eruption, on the POSS red chart (copyright: National Geographic Magazine-Palomar Observatory Sky Survey).

to be ~ 0.5 mag. From these magnitudes the star is unlikely to be a giant since it would then lie at a distance of at least 15 kpc which would place it far from the plane and pose difficulties in reconciling the observed X-ray flux with the Eddington limit⁴ if the X-ray source has a mass similar to that of the Sun. If it is a K dwarf the estimated colour would demand some obscuration, presumably circumstellar perhaps arising from earlier outbursts⁷, and a distance of the order of 500 pc. Such obscuration would be of interest in view of reports⁸ of interstellar lines. In addition, the magnitude expected⁴ from extrapolating the X-ray spectrum near maximum to the optical region as an optically thin bremsstrahlung source, is $B \sim 8$, which may also indicate the presence of interstellar absorption, although it could also represent self-absorption in the hot gas emitting the X-rays.

If the optical counterpart before eruption is a red dwarf star, this is consistent with a model for the X-ray source resembling current explanations of dwarf novae (see for example ref. 9), but with a black hole or neutron star replacing the white dwarf as the body accreting material spilt from the Roche lobe of the red star.

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Galactic superhaloes, globular clusters and high latitude X-ray sources

I show here that the observational features of unidentified, high latitude, X-ray sources¹ are consistent with the suggestion that they are a population of remote, optically undetectable, globular clusters. The principal objection to the hypothesis that the high latitude sources are members of any galactic population is their isotropy in galactic longitude. But if it is assumed that the most distant unidentified high latitude source radiates at 10^{38} erg s⁻¹ (a typical value for a bright globular cluster X-ray source^{2,3}) and that it is the faintest unidentified high latitude source, visible at 1-2 Uhuru counts s⁻¹, its distance would be ~ 200 kpc. Then, extrapolating to the brightest unidentified source at 7 counts s⁻¹, we get a lower bound on the minimum distance to any objects of about 80 kpc, and the volume toward the galactic centre, between $-90^\circ < l'' < 90^\circ$ in the 'shell' from 80 to 200 kpc, differs negligibly from the volume toward the anticentre, at $90^\circ < l'' < 270^\circ$. Although a specific radiation model is used in this case, the luminosities do not affect the basic result.

It is widely believed that the globular cluster population of the Galaxy is essentially completely observable⁴; however, that may not be the case. Table 1 shows concentration class

Table 1 Average absolute photographic magnitude of different classes of globular clusters*

Class	M_{pg}	Class	M_{pg}
I	-7.9	VII	-7.5
II	-7.8	VIII	-7.2
III	-7.8	IX	-6.7
IV	-7.8	X	-6.2
V	-7.7	XI	-5.7
VI	-7.6	XII	-5.2

*Data from ref. 5.

against average absolute photographic magnitude for 91 clusters⁵, and it can be seen that as the cluster gets more diffuse (higher number class), the integrated absolute magnitude falls. Indeed, out to an apparent value of $m_{pg} = 15$, all clusters are known only to concentration class X or tighter⁴. And, in fact, 10 of the 12 'new' globular clusters detected in the Palomar Sky Survey plates are in class XI or XII (ref. 6). These new clusters are difficult to detect because of three characteristics: they are intrinsically fainter than the others, they are diffuse (and thus more difficult to discriminate from the general field), and they are far away.

Pal 3 and Pal 4 have been examined photometrically⁷. Those particular clusters are about 100 kpc away, and it is possible that because of their faint absolute magnitude, such objects may populate space in great numbers and remain undetected⁷. The average B magnitude of the 25 brightest stars in these clusters is $\sim 20^4$; even Pal 12, 33 kpc away, has an average B magnitude of 17.5 for its 25 brightest stars. These faint magnitudes, and the diffuse nature of the clusters make it likely that the NGC and IC catalogues would not include other similar objects⁴. Since the limiting red magnitudes of the Palomar plates are ~ 20 ,—assuming that the red giant stars have $M_v = 0.0$ with the total integrated cluster magnitude $M_v = -6$ (ref. 7; see also Table 1)—then all clusters fainter than $m_v \approx 14$ will probably be undetectable. Pal 3 and Pal 4 have m_v values of 14.2 and 14.4, respectively⁷.

The difficulty of detecting distant faint clusters is demonstrated by Fig. 1. Clearly, the visible globular clusters are on the edge of detectability.

The distribution of the number density of the globular clusters against the distance from the galactic centre^{4,8} describes well the spatial density of the nearer clusters, but that is not so at large distances. Figure 2 shows the number of globular clusters in 10 kpc shells, against the distance from the galactic centre, for all clusters whose distances are known¹.

Further, 6 globular clusters have been observed at > 80 kpc and none at all have been observed between 50 and 80 kpc. To explain these observations, I propose that the density distribution of globular clusters changes, becoming roughly proportional to the volume element at a point in space 50–80 kpc from the galactic centre. With such a model a preponderance of clusters could be expected at about the limit of detectability, since beyond that point the clusters would not be visible by definition, whereas at closer distances the volume surveyed and thus the number of objects observed would be smaller.

Approximately 150 unidentified, high latitude, X-ray sources have been observed down to ~ 1.5 Uhuru counts s^{-1} (after coverage corrections)¹. If it is assumed that X-ray sources occur in these remote, diffuse clusters in the same ratio—4/119—as in the nearer clusters², about 4,500 class XI or XII globular clusters would have to be present between roughly 80 kpc, where the first 'distant' clusters are observed, and the limit of 200 kpc. Assuming a constant space density gives: $\rho = 1.3 \times 10^{-4}$ clusters kpc^{-3} . That is almost exactly equal to the space density of globular clusters between 20 and 30 kpc; and 4,500 clusters with an initial mass of $\sim 10^5 M_{\odot}$ are still much less massive than the Galaxy.

The possibility that high velocity stars form 'moving groups', belonging to the halo population, has been discussed^{9,10}, and it has been suggested that these groups may share an equality of motion because they are remnants of globular clusters. It has been estimated that the number of parent globular clusters is of the order of 10^3 (ref. 10).

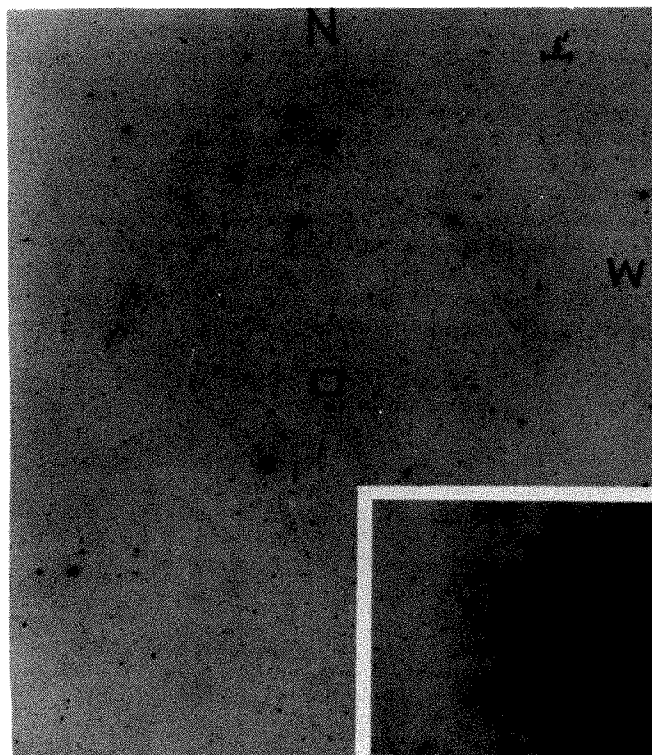


Fig. 1 Region around Pal 4, a remote globular cluster. Inset, 200'' plate of the same object taken by Burbidge and Sandage⁷ (covering the area of sky boxed in the main figure). Scale bar, 6'; inset scale bar, 60''.

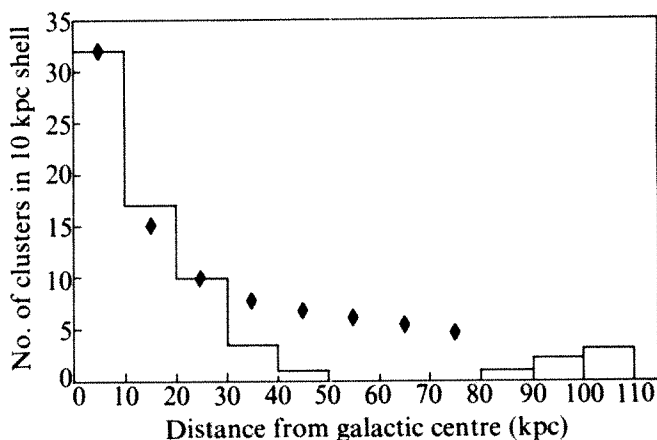


Fig. 2 Number of globular clusters in 10 kpc shells against distance from the galactic centre. ♦, Predicted values based on $\rho \propto r^{-2.7}$ normalised to the value observed between 0 and 10 kpc; χ^2 per degree of freedom is 0.8 out to 40 kpc, and 3.3 out to 80 kpc. For a $\rho \propto r^{-2.9}$, χ^2 is 1.4 and 2.4 out to 40 kpc and 80 kpc, respectively.

The observational implications are clear: searches should be made for variability, and the positional accuracy of the high latitude X-ray sources should be improved. Also, deep sky plates in the red should be made. Further distance estimates to clusters for which information does not already exist would help in understanding the true density distribution function. If, indeed, the unidentified high latitude sources are stellar objects in a superhalo population of diffuse globular clusters, then the log N –log S curve¹ should flatten beyond the true edge of the halo.

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Gas well pressure fluctuations and earthquakes

AN important part of earthquake prediction studies is the search for various precursors. It is likely that one precursor may not enable us to predict an earthquake correctly, but a collection of these may remove the uncertainties. It is also desirable to find data that are recorded routinely, in enough detail, such that they can be conveniently used by seismologists.

In so far as the permeability (due to pores and/or joints) and the pressure gradient in a reservoir may be influenced by stress, production and well-head pressure data from oil and gas wells may prove to be useful. There has been an attempt to search for precursory phenomenon in monthly oil well production data¹. On the other hand, many examples exist already of changes of levels and flow rates in water wells before and after earthquakes^{2,3}; it is reasonable to expect that oil and gas wells would behave in much the same way.

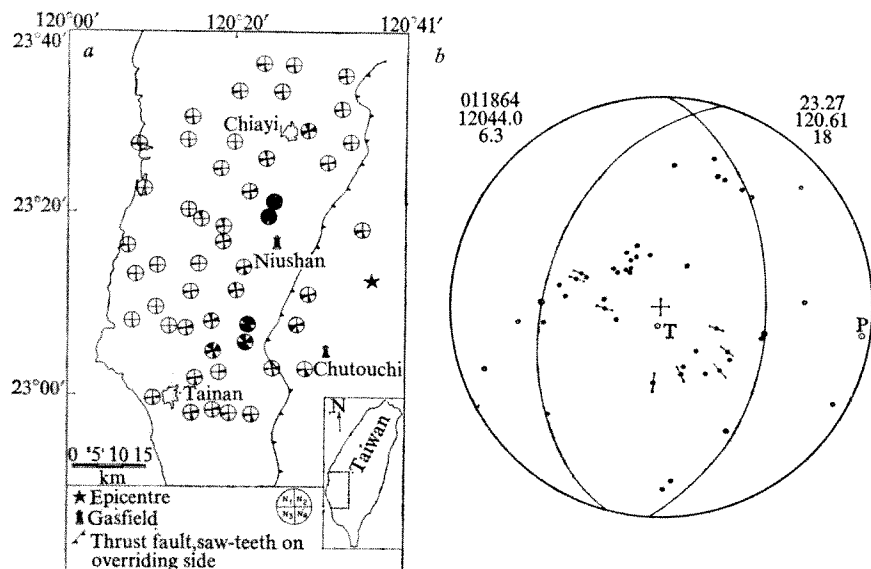


Fig. 1 *a*, The location of the earthquake epicentre, the causative fault and the Niushan and Chutouchi gas fields. The damage figures are indicative of the extent of the source region to the west of the fault, where the towns (indicated by circles) are of approximately equal size in terms of population and number of structures. To the east of the fault, in the mountainous area urbanisation is much less dense. The numbers N_1, N_2, \dots are normalised to the maxima. N_1 , no. of dead; N_2 , no. of wounded; N_3 , no. of houses collapsed; N_4 , no. of houses damaged. *b*, The lower hemisphere equal area projection of the focal mechanism of the January 18, 1964 earthquake. T, tension axis; P, compression axis.

Here we report a case of pressure or production changes in two gas fields in Taiwan before a 6.75 magnitude (Pasadena M_s) earthquake.

Figure 1*a* shows the location of the Niushan and Chutouchi gas fields, the epicentre and the extent of damage. This region is very active seismically and since the mid-1600s, when historical records became available in this area, many destructive earthquakes have occurred here. The earthquake of January 18, 1964 (origin time 1204:40.0 GMT; depth 17 km (W. H. K. Lee and J. P. Yang, unpublished)) is apparently related to an east dipping thrust fault mapped by surface as well as subsurface geological data. The fault plane solution is shown in Fig. 1*b*. From the magnitude of the event and the damage pattern (Fig. 1*a*), the fault length associated with this earthquake is of the order of 15–35 km (ref. 4); the Niushan field is within 8 km and Chutouchi field 12 km of the surface trace of the causative fault and are therefore within the source region of the earthquake.

Niushan gas field produces from upper Pliocene units that are composed of alternating beds of sandy shale, silty sandstone, shale and fine to medium grained sandstone. Chutouchi field produces from lower Miocene fine compact sandstone and badly sorted silty sandstone. In both fields the porosity is low and the gas was produced from jointed and fractured rocks⁵.

Both gas fields were in operation between 1957 and 1970. Of the more than ten wells in Niushan gas field, only three were not pumped. Ideally both well-head pressure and production rate data for such wells are useful, since both are related to the change in permeability and/or pressure gradient in the reservoir resulting from tectonic stress. The production data in Niushan field are not usable, however; apparently the total production of the field, including pumped wells, is correct, but the individual readings were rough estimates.

Because of the presence of water in the well and the bubbling of gas, the pressure varies between daily maxima and minima; the pressure is recorded continuously, but only the maxima and minima were entered in the production records which were used in this work. In Fig. 2, the daily maxima and minima since January 1, 1964 are plotted for the three wells. There are rather clear increases (15–60%) in pressure for all three wells for both daily highs and lows, starting from January 8. Careful examination of records covering several years shows that such rapid change did not appear; the pressure readings have short term variations of $\pm 0.1 \text{ kg cm}^{-2}$ superposed on a very slowly decreasing trend for several years before the earthquake. After the earthquake, there was a noticeable short term fluctuation in one of the wells, but not in the other. It should be noted that a break in the piping system occurred during the earthquake resulting in open flow of the wells and an ensuing drop in well

pressure. The pressure returned to the level existing before January 9 after the pipes were repaired on January 20, although one of the wells (R-10) was permanently damaged during the earthquake.

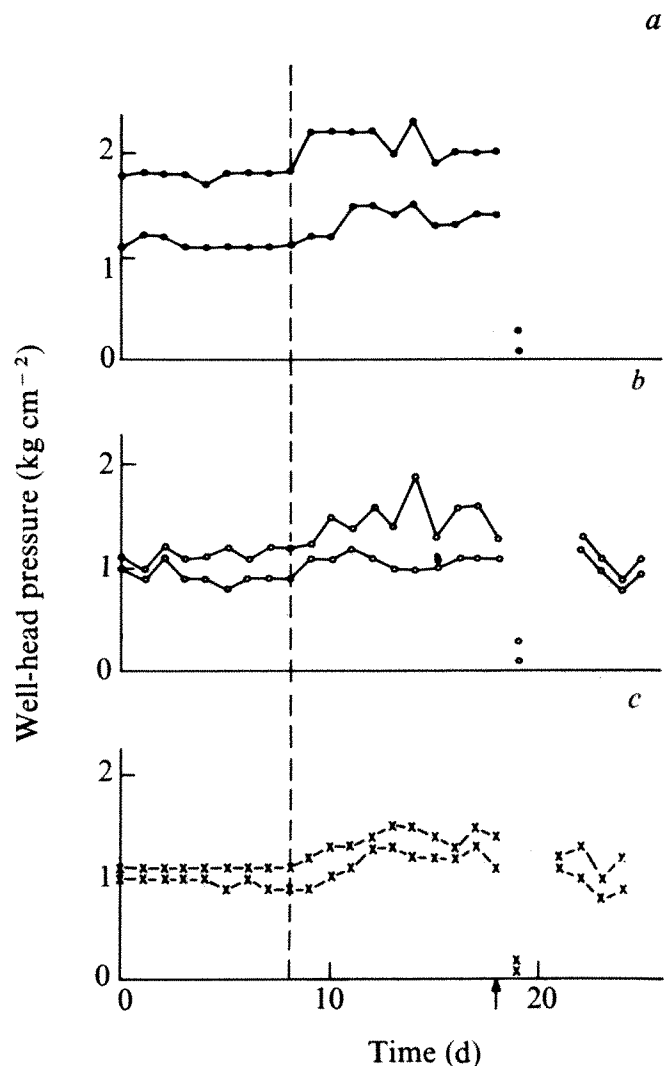


Fig. 2 The well-head pressure variation before and after the January 18, 1964 earthquake (arrow) at Niushan gas field. *a*, Well R-10, depth 988.6 m; *b*, well R-7, depth 717 m; *c*, well R-23, depth 385 m.

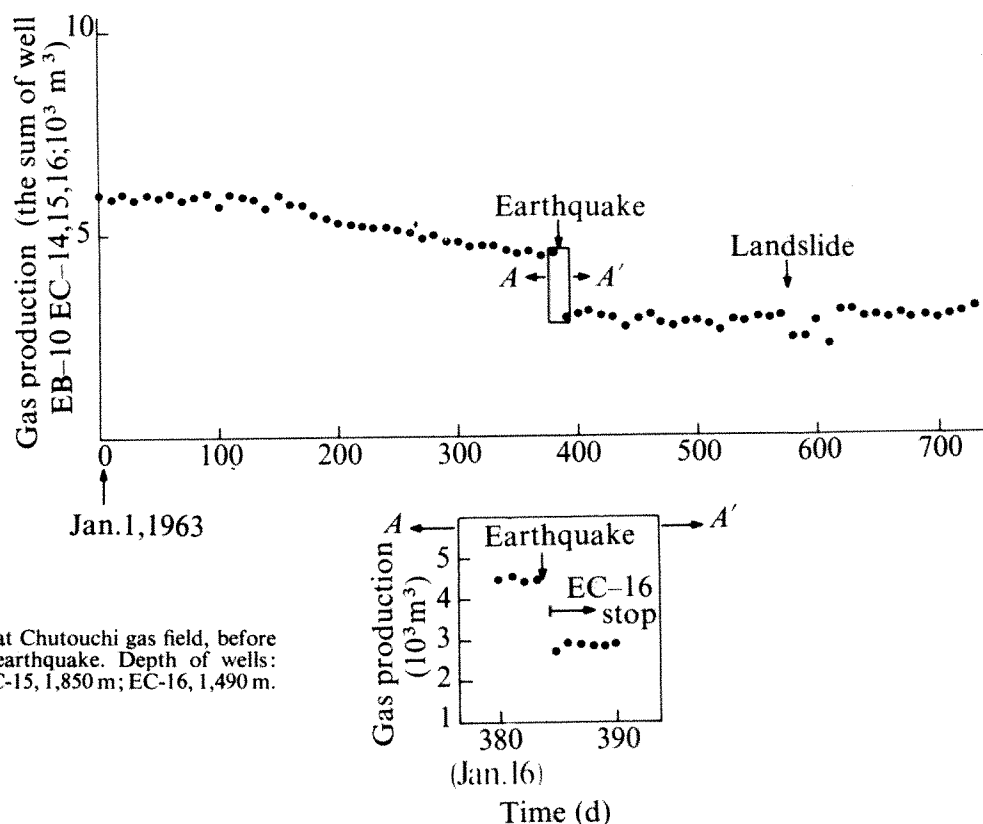


Fig. 3 Gas production rate data at Chutouchi gas field, before and after the January 18, 1964 earthquake. Depth of wells: EB-10, 1,440 m; EC-14, 1,324 m; EC-15, 1,850 m; EC-16, 1,490 m.

The increase in pressure before the January 18 earthquake is apparently a precursor to the earthquake.

At the Chutouchi gas field, on the hanging wall side of the thrust (Fig. 1), the pressure and production rate showed only slight changes during the period of January 1–18. We then looked at the production rate data for all three wells from 1958 and found that superposed on a general long term slow decrease, there is a noticeably more rapid drop beginning after March 1963 (Fig. 3); after the earthquake the trend became steady again. (The drop in total production after the earthquake was caused by the disruption of one well during the earthquake.)

The difference in well pressure behaviour at these two fields before the earthquake may indicate the different behaviour of reservoirs under stress on two sides of the causative fault; Niushan gas field is on the foot wall side and Chutouchi field on the hanging wall side. The exact mechanism of this behaviour can be postulated only after the stress field around the fault and the joint system are known in detail.

As demonstrated by Myachkin and Zubkov⁶, earthquake precursors can be classified into short term and long term. The Niushan pressure change can be classified as a short term precursor, although the time interval between the commencement of this precursory change and the occurrence of the earthquake is an order of magnitude longer than most of the results of Myachkin and Zubkov (see figures in ref. 6). On the other hand, if the Chutouchi data are a true precursor, then they are closer to being a long term precursor.

Because the pressure and production data are continuously monitored by the oil companies, they may be a worthwhile source of evidence of the existence and usefulness of this precursor in other gas and oil fields.

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Gravitational drive on oceanic plates caused by thermal contraction

PROPOSED mechanisms for the motion of lithospheric plates over the Earth's surface range from the self-drive of plates by gravitational sliding^{1,2}, through passive response to mantle-wide convection³, to point drive by upwelling hotspots in the mantle⁴. All of these models depend on the distribution of effective viscosity within the mantle. Investigations⁵ have established the strong likelihood that a weak layer (10^{20} poise) exists immediately below the lithosphere, and long spatial-wavelength loadings associated with changes in the Earth's rotation⁶, together with the larger scale components of glacial loading⁵, demand that this asthenosphere be quite thin (100 km) and bounded by material from the lower mantle with a viscosity of two to four orders of magnitude higher.

As an analogy, this situation can be visualised as a layer of ice, over a layer of water, over a layer of honey 30 times as deep. Thus it can be seen that the plates (the ice in this analogy) would not be well coupled to motions in the lower mantle (the honey), and, indeed, that the highly viscous lower layer would be unlikely to flow faster than the surface layer. Several observations support this concept. Morgan⁷ has constructed plate histories that require very little relative motion between hotspots, and there is significant coincidence between the hotspot frame of reference and a frame of reference derived by minimising the motion of plate boundaries⁸.

The boundary-layer theory of plate accretion has received strong support from the discovery of the square-root law of

implied by this relationship, and the material must be assigned a constant volume expansion coefficient, γ , for the calculation of $\rho(z)$. Then

$$\rho = \rho_A \gamma (T_1 - T) = \rho_A \gamma T_1 \operatorname{erfc} [z/2\sqrt{(\kappa t)}]$$

giving h and, therefore, α (see ref. 9):

$$h = [1/(\rho_A - \rho_w)] \int_0^\infty \rho dz = (1/\sqrt{\pi}) [\rho_A \gamma T_1 2\sqrt{(\kappa t)} / (\rho_A - \rho_w)]$$

$$\alpha = \frac{dh}{dx} = \frac{1}{s} \frac{dh}{dt} = \frac{1}{\sqrt{\pi}} \frac{\rho_A \gamma T_1}{s(\rho_A - \rho_w)} \sqrt{(\kappa/t)}$$

where s was the spreading velocity of the lithosphere when it was formed, and $\beta - \alpha$ may be found from the slope of the locus, $l = 2y\sqrt{(\kappa t)}$ (constant y):

$$\beta - \alpha = \frac{dl}{dx} = \frac{1}{s} \frac{dl}{dt} = \frac{y}{s} \sqrt{(\kappa/t)}$$

thus, equation (4) becomes:

$$\begin{aligned} \frac{dF}{dx} - \tau = \frac{g\rho_A \gamma T_1 \kappa}{s} \left[\frac{1}{\sqrt{\pi}} + \left(1 + \frac{\rho_A \gamma T_1}{y\sqrt{\pi}(\rho_A - \rho_w)} \right) \times \right. \\ \left. \times \left(\frac{1}{\sqrt{\pi}} (1 - e^{-y^2}) + y \operatorname{erfc} y \right) - 2 \left(\frac{1}{\sqrt{\pi}} (1 - \frac{1}{2} e^{-y^2}) - \frac{1}{4y} \operatorname{erfc} y + \frac{1}{2} y \operatorname{erfc} y \right) \right] \end{aligned}$$

where it should be noted that the integrals of the error function differ from the standard forms because of integration from 0 to y instead of from y to ∞ . Finally, this simplifies to:

$$\begin{aligned} \frac{dF}{dx} - \tau = \frac{g\rho_A \gamma T_1 \kappa}{s} \left[\operatorname{erfc} y + \frac{2\rho_A \gamma T_1}{\sqrt{\pi}(\rho_A - \rho_w)} \times \right. \\ \left. \times \left(\frac{1}{\sqrt{\pi}} (1 - e^{-y^2}) + y \operatorname{erfc} y \right) \right] \quad (5) \end{aligned}$$

the second term of which can be neglected for reasonable values of y , γ and T_1 . For example, ridge topography has been⁹ well fitted by using $\rho_A = 3.3 \text{ g cm}^{-3}$; $\gamma = 4 \times 10^{-5} \text{ }^\circ\text{C}^{-1}$; $\kappa = 8 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$; $T_1 = 1,120 \text{ }^\circ\text{C}$ (Pacific Ocean). The parameter γT_1 is, therefore, 0.045, and the choice of y is not critical as is shown in Table 1.

Presuming that asthenosphere material becomes essentially rigid after a relatively small drop in temperature, the final result can be written simply as

$$dF/dx - \tau \approx g\rho_A \gamma T_1 \kappa / s = 7.2 \text{ bar} \quad (s = 5 \text{ cm yr}^{-1}) \quad (6)$$

the most startling feature of which is that it is independent of the age of the lithosphere. For comparison of figures, the drag on lithosphere moving at 10 cm yr^{-1} over 100 km of asthenosphere of 10^{20} poise, is 3 bar. In the absence of such drag, the lateral compressive stress developed over 5,000 km of plate 100 km thick at its end is $50 \times 7.2 = 360 \text{ bar}$.

The conclusions this analysis points to are both simple and important. The overall magnitude of the driving force is similar to that derived by some authors^{10,12} but not to that derived by Jacoby¹. There is no source for the high compressive stresses invoked by Watts and Talwani¹³ to explain the topographical bulge and high gravity on the seaward side of many trenches. The thermal contraction force is independent of the age of the lithosphere and is only dependent on the velocity at which it was originally spread. It is not an edge

Table 1 Variation of parameters with y

y	0	1	2	3
$T/T_1 = \operatorname{erfc} y$	0	0.843	0.995	0.99998
$h \propto \int_0^y \operatorname{erfc} y$	0	0.524	0.563	0.564

force associated only with active mid-oceanic ridge crests, as assumed by Forsyth and Uyeda¹⁴. This removes one of the two arguments advanced by McKenzie¹² against plate drive by ridges: that is, small plates should not necessarily move faster than large plates. Another argument¹² is that the mechanical work produced by the ridges is insufficient to account for the elastic energy released in earthquakes. This is easily refuted by the observation that the majority of energetic earthquakes are associated with subduction zones. More mechanical work is generated by sinking lithosphere than by gravitational sliding from ridges, but this work is not necessarily available to drive the plates. Analysis based on the correlation of plate movement with boundary composition¹⁴ shows that the subduction zones contain both the largest driving force and the greatest resistance to flow. Although only edge drive by ridges is permitted, the conclusion arises inexorably from the fact that all fast moving plates are bounded by trenches. Unfortunately, this may merely reflect the inability of any fast-moving plate to override another without generating a huge resistance.

The thermal contraction drive is ample to push plates over any asthenosphere that complies reasonably with the known constraints, and the presence of subduction zones may exercise indirect control over plate motions by providing the only available low resistance against the forward end for fast moving plates. A more serious objection to drive by thermal contraction is the fact that in large mountain ranges the lateral stresses in the lithosphere would have to be about three times greater than can be provided by ridges^{2,10}. The answer to this objection is, in part, similar to that to the problem of earthquake energy: the largest mountain ranges are associated with sinking slabs, present or past. The excess hydrostatic pressure at shallow depth under an isostatically compensated mountain range can be partially balanced by a pressure deficit at intermediate depth if the region is underlain by anomalously dense mantle. An extra oceanic slab, slung flat beneath a mountain range, can provide a regional 'suction' equal to the drive provided by the ridge, and that is increased further in the extra mountain 'root' region needed to compensate for the slab. Long tilted slabs can create even larger 'suction' forces. The other class of substantial mountain ranges, associated with continental rift zones, are probably uplifted by thermal expansion and magma buoyancy¹⁰. Since they are rifting, compensatory horizontal compression is not required.

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Coated optical guides for spectrophotometry of chemical reactions

A NEED exists for a method of detecting small amounts of chemicals in air and other gases, and in solutions, simply, and with a high degree of sensitivity. We have developed a new technique based on the concept of using waveguide phenomena in combination with chemical reactions. This technique is applicable to a wide variety of chemical reactions, including biochemical systems, provided that they cause a change in light transmittance by virtue of a change in colour, refractive index, or light scattering. Both real-time and cumulative measurements are possible.

The theory underlying light guide phenomena is well understood and has been treated extensively by Kapany^{1,2}. We felt it should be possible to alter the transmission properties of an optical guide using a chemical reaction on the surface of the guide, since a change in refractive index at the interface with the rod surface alters the critical angle. Initially, reactants were applied directly to a rod which functioned as a lightguide. Changes in light intensity were observed qualitatively as a

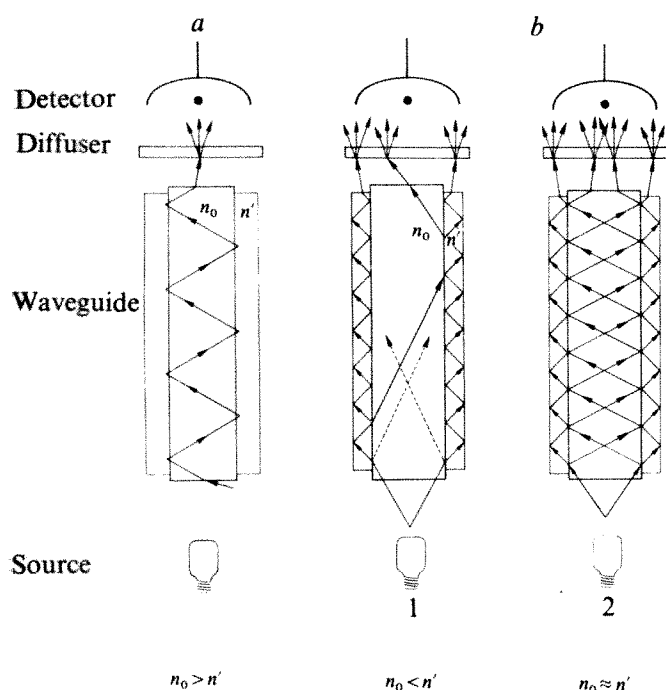


Fig. 1 Mechanism models.

reaction was carried out on the surface. But problems in coating adhesion were encountered using this technique. Consequently, a solution of water-soluble polymer and reactants was prepared, which was then used to coat a 1.0×20 mm quartz rod. Thus, the polymer acted as a bonding agent and matrix, and also offered protection to the reactants incorporated in the coating.

The lightguide coating combination can be chosen to provide a coating with a refractive index that is either higher or lower than the rod (guide) and which can be either a water or non-water based polymer, depending upon its compatibility with the desired chemical reactants. A lower refractive index is normally used in optical guide applications and would result in the mechanism illustrated (Fig. 1a).

Using the higher refractive index coating, the mechanism shown in Fig. 1b would be operative. Although either approach can be used, the mode shown in Fig. 1a would result in lower sensitivity since the evanescent wave interactions occur only in the region of the rod-coating interface. In either of the mechanisms shown in Fig. 1b, the radiation is transmitted

through the entire coating and so allows solid state spectrophotometric measurements *in situ*.

To measure the light transmittance, an instrument was designed and constructed to provide quantitative analytical measurements. This 'gradient light analytical detector' (GLAD) will accommodate glass rods of 0.9–1.3 mm in diameter, and either 10 or 20 mm long (Fig. 2).

A detailed view of the GLAD optical system is also shown in Fig. 2, with rod dimensions exaggerated to illustrate the basic instrumental operation. The substage condenser converts the collimated beam into a strongly converging hollow cone of light. The hemispherical lens and circular aperture couple the light into the rod. After multiple reflections within the rod, the light emerges at the upper face and is scattered by a diffuser, part of the light going into the silicon photodiode detector. The photodiode is operated in the photovoltaic mode, the operational amplifier acting as a current sink to minimise the voltage across the diode. The amplifier output is a low impedance voltage, proportional to the input current over a range of 10^{-11} – 10^{-3} A.

To demonstrate the concept, we used the technique for the determination of microgram quantities of sodium cyanide in a cyanide-picric acid system. A solution was prepared to contain 1% by weight of polyvinyl alcohol and 0.1% by weight of sodium picric acid, which is known to respond selectively to $(CN)^-$ (ref. 3). This solution was then used to coat the surface of the rods uniformly when the ends were protected.

The light transmission of the dry, coated lightguides was measured before reaction. Known amounts of cyanide ion, in the form of sodium cyanide, were applied to the outer surface of the guides using a 5 μ l Eppendorf pipette, the rods were dried, and the transmission was measured again. The percentage transmission, based on the initial reading before exposure, was then plotted as a function of the $(CN)^-$ concentration (Fig. 3).

The reaction between the cyanide ion and the picric acid changes the refractive index and absorption coefficient of the coating. The resulting change in light transmission through the guide is proportional to the concentration of the cyanide species. The multiple internal reflections enhances the sensitivity, in that a small change in the optical characteristics of the coating

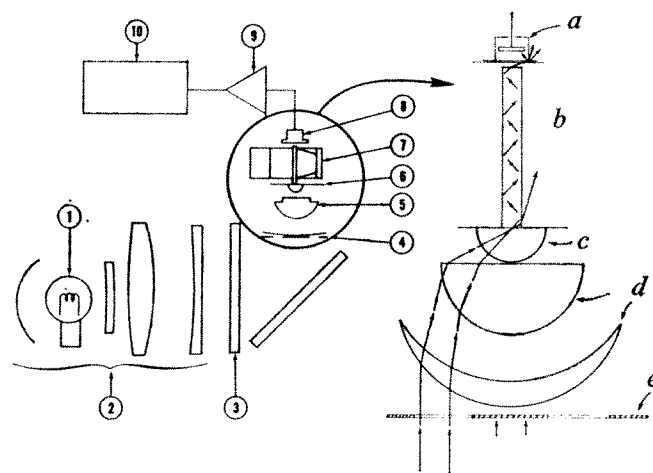


Fig. 2 Block diagram of the gradient light analytical detector (GLAD). 1, A tungsten filament lamp light source; 2, a condenser system to produce nearly collimated light; 3, a filter for wavelength selection; 4, an annular aperture to block axial light rays; 5, a condenser to produce a hollow cone of light rays; 6, coupling hemispheres and apertures to couple large angle rays into the rod; 7, a rod mount to position rods accurately with respect to the aperture while presenting a minimum of surface contact; 8, a silicon photodiode detector; 9, an operational amplifier operating as a 'current-voltage' converter; 10, a three-digit digital voltmeter for relative transmittance readout. a, Detector; b, rod; c, hemisphere; d, condensers; e, annular aperture.

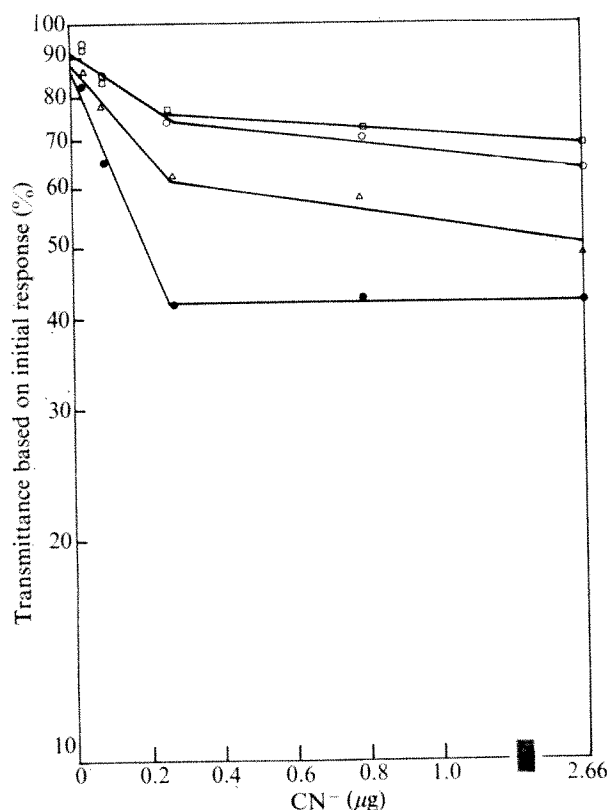


Fig. 3 Concentration of $(CN)^-$ against transmittance. \square , Red filter; \circ , achromatic light; \triangle , orange-yellow filter; \bullet , green filter.

causes a large change in the light transmitted through the system. A green filter provides the best sensitivity, as can be anticipated because of the reddish-brown colour of the reaction product; further, Beer's law is obeyed over the expected range of concentration.

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Implications of drought for vertebrate fossil assemblages

THE profound effects of drought on modern ecosystems is widely appreciated. However, little attention has been paid to the palaeontological and geological consequences of drought; the few works¹ which discuss drought in these terms are general. I wish to describe a sequence of events that may occur during a drought and the taphonomic implications of these events.

It is well known that factors such as habitat preference of the animals in question^{2,3}, the degree of disruption of bones by

carnivores⁴, and the environmental setting⁵ influence the likelihood of preservation of thanatocoenoses. My preliminary observations of the Ethiopian drought during 1973 indicate that these factors and many others are affected considerably by drought. I suggest that assemblages of vertebrates that die during a drought may have a disproportionate probability of being represented in the fossil record and that such assemblages can be recognised by the characteristic alterations of an ecosystem by drought.

Tannehill⁶ offers a useful definition of drought as a period of deficient rainfall which is seriously injurious to local vegetation. He avoids defining drought in terms of the amount of rainfall, since what is normal rainfall in one area may be deficient in another. I describe three hypothetical phases of progressive severity of drought. Although the effects of a normal dry season may be the same as some of those described for these phases, it is the complex of effects that indicates an abnormal period of dryness—drought—as opposed to a normal dry season.

In phase I, mild drought, rainfall is less than usual. New vegetation withers quickly or fails to grow; large migratory herbivores move in search of better pastures and more water; seasonal waterholes and streams dry up; mortality is elevated above normal attritional levels, especially among younger individuals.

In phase II, severe drought, rainfall continues to be deficient. Permanent rivers and lakes are shrinking; caliche forms in the alkaline soil, evaporites precipitate and cracks develop in the beds of dried-up rivers and waterholes. Animals respond to the drought according to the frequency with which they need to drink water; thus highly mobile animals with moderate water needs may migrate out of the area. Those animals that need to drink at least once a day congregate along the remaining waterways regardless of their normal habitat preference. All edible vegetation within a critical distance of water is soon eaten, so only those animals that rarely drink water can travel far enough from water to find forage. Many animals die of starvation near major water sources. I observed numerous carcasses within 50 m of the Awash River in Ethiopia; thus the presence of a river or lake near a fossil assemblage in no way indicates the absence of drought. Carnivores may migrate into the area because of the easy availability of food. Observations of hyenas in the Awash suggest that even carnivores which habitually consume bones alter their habits during a drought and eat soft tissues instead; thus skeletons are rarely disturbed by the carnivores during this phase.

In phase III, extreme drought, normal rainfall is still absent. Large lakes and rivers may dry up, leaving huge numbers of dead fish, amphibians and other water-dwelling species. The few herbivores crowd around shrinking pools of water and die struggling to drink. Local extinction of plants and animals is common; extinction may occur on the species level as well. All but the most drought-resistant plants have died. The soil moisture and organic content are low; loose sand may be blown into dunes. If phase III continues long enough, the environment may be permanently altered into desert or semi-desert; in this case, the situation is no longer one of drought. The plants and animals which originally inhabited the area do not return and colonisation by organisms adapted to desert conditions occurs.

For several reasons, a large runoff is likely when rain recurs following phase II or III. The land surface is denuded of vegetation that normally traps and holds water. Caliche impedes the passage of rainwater into the soil, further increasing runoff. Both intermittent and permanent rivers may fill and overflow, transporting and depositing large quantities of sediment. Skeletons lying in or near river or lakebeds are covered rapidly by these sediments; fossilisation may begin.

So far, the few drought assemblages that have been recognised fall into phase III (ref. 7), probably because the effects of this phase are more easily recognisable than the more subtle effects of phase I or II. Phase I assemblages are difficult to distinguish from assemblages resulting from other catastrophes. Phase II

droughts, however, alter the ecosystem sufficiently to be identified. Since failure to recognise a phase II assemblage may seriously distort a palaeoecological reconstruction, it is important that phase II assemblages be distinguished. The following criteria may be used to identify phase II assemblages except those criteria specified as characterising phase III assemblages. No single criterion should be considered as diagnostic in itself, nor will every phase II assemblage exhibit all of these criteria.

The palaeontological criteria are as follows. (1) Since many animals die of starvation rather than thirst, they are buried close to major water sources. (2) The age distribution of individuals killed by drought is indicative of catastrophic mortality¹⁻³. Many young individuals die in phase I; those dying in phase II are likely to be in their prime. (3) Many skeletons are preserved partially or fully articulated because carnivores have not disturbed the bones. Because the palaeo-environment lacks moisture, soft tissues of carcasses are dried and help to keep joints intact⁹. (4) Animals of disparate habitat preference are found in association. Most species are highly dependent on water. (5) In phase III, aquatic or semi-aquatic animals die concentrated in small areas. (6) In phase III, animals such as crocodiles and lungfish die during aestivation. The geological criteria are as follows. (1) Fine-grained strata associated with the fossils show mud cracks. (2) Evaporites and caliche are commonly associated with the fossils but are uncommon in other strata of the deposit. (3) Overlying sediments indicate rapid deposition.

Further study of modern droughts will undoubtedly modify these criteria. Even in their present form, however, they may be useful in re-examining previously described fossil assemblages for which other causes of death have been proposed. On reconsideration, assemblages that include numerous herbivores found in death poses and located near major water sources—such as the *Leptomeryx* assemblages described by Clark and Guensberg¹⁰—may prove to be drought assemblages. Although it has been speculated before⁷ that drought may be related to the extinction of some species, these criteria will enable more certain identification to be made of such extinctions. Finally, if the geological criteria are correct, palaeontologists may be able to find new sites by surveying areas near water sources and characterised by caliche, evaporites and mud cracks.

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Hypsilophodon and *Iguanodon* from the Lower Cretaceous of North America

WE describe here ornithopod dinosaur remains from the Lower Cretaceous of western North America, which can be referred to the European genera *Hypsilophodon* and *Iguanodon*. The occurrence of these remains indicate that a land connection existed between Europe and North America at the end of the Jurassic.

Though *Hypsilophodon* and *Iguanodon* are well known from Europe and northern Africa (Fig. 1a) no trace of either genus has yet been reported from North America and the only possible Neocomian ornithopod described from that continent is from the Lakota Sandstone of South Dakota and is referred to *Camptosaurus*¹, a genus well known from the older Morrison Formation (Upper Jurassic). *Camptosaurus*¹ is very different from both *Hypsilophodon*² and *Iguanodon*³. No material of *Iguanodon* is identifiable in the extensive collections from the Morrison Formation of central North America and all the hypsilophodontid material is referable to two genera⁴, *Laosaurus* and *Dryosaurus*. An extensive dinosaur fauna is known from the Cloverly Formation of Montana and Wyoming⁵ but this is younger (Aptian–Albian) than the Wealden, and the iguanodontid *Tenontosaurus*⁶, the only Cloverly ornithopod described,

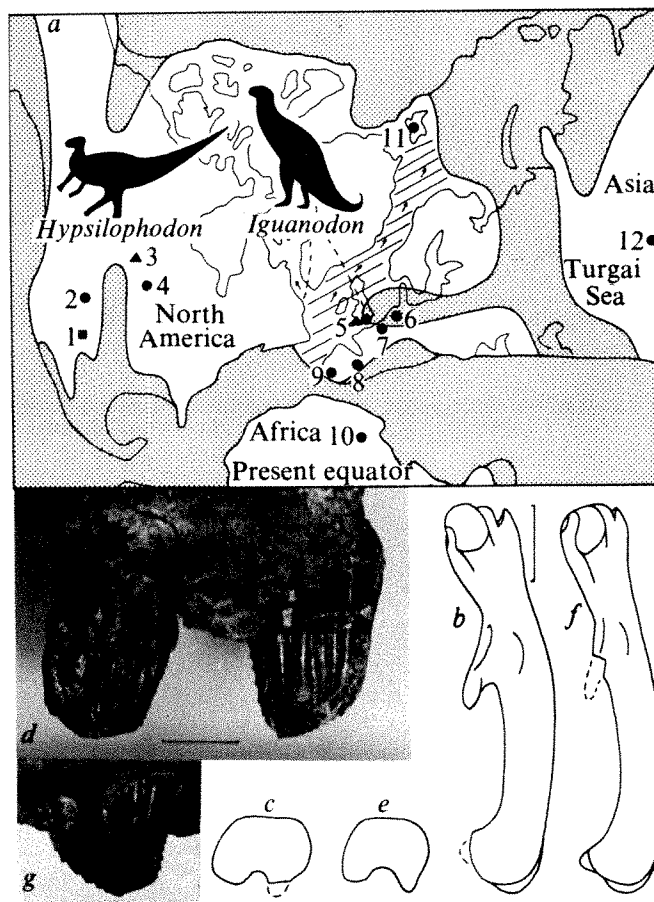


Fig. 1 a, Localities and distribution of land in part of the Northern Hemisphere during the early Cretaceous, after Cox¹⁷, — reconstructed coast lines; —, present coast lines; - - -, northern extent of North Atlantic, after Dietz and Holden²²; →→→, circulation currents, after Berggren and Hollister²¹; cross hatching, epicontinental sea, after Hallam²⁰; ▲, *Hypsilophodon* localities; ●, *Iguanodon* localities; ■, *Tenontosaurus* locality; 1, southern Arizona; 2, south-eastern Utah; 3, western South Dakota; 4, eastern Nebraska; 5, southern England; 6, Belgium; 7, France; 8, Spain; 9, Portugal; 10, Tunisia; 11, Spitzbergen; 12, eastern Gobi Desert (shifted slightly left). b and c, left femur ($\times 0.2$) of *Hypsilophodon* sp. (American Museum of Natural History No. 2585), collected in Lakota Sandstone about 38 m above the Morrison Formation 4.8 km north of Piedmont, South Dakota; b, medial view; c, distal end; d, lateral view of posterior part of right maxilla ($\times 1.0$) of *Iguanodon* sp. (Brigham Young University Earth Sciences Museum No. 2000), collected 1.2 km east of Dalton Well, Grand County, Utah; e and f, left femur ($\times 0.5$) of *Hypsilophodon foxii*, Wealden of Isle of Wight; e, distal end; f, medial view; g, lateral view of posterior tooth of left maxilla⁹ ($\times 1.0$) of *Iguanodon mantelli* (British Museum (Natural History) No. R754), from Wealden (Tilgate Forest) of Cuckfield, Sussex. Scale lines represent 5 cm (b, c, e, and f) or 1 cm (d and g); although the North American *Hypsilophodon* femur is much larger than the European specimen figured here, it falls within the size range of the genus⁴.

is only distantly related to *Iguanodon*. Consequently, the specimens described here (Fig. 1b, c and d) represent the first material of the characteristically European genera *Hypsilophodon* and *Iguanodon* to be recognised from North America.

The left femur (Fig. 1b and c), from the Lakota Sandstone of South Dakota, is very similar to the femora of *Hypsilophodon foxii*² (Fig. 1e and f). The other hypsilophodontid femora from North America differ from these in several respects. In *Hypsilophodon* itself there is a shallow cleft separating the proximal trochanters (Fig. 1b and f) whereas in *Laosaurus* (= *Nanosaurus rex*)⁸ and *Dryosaurus* the cleft is deep; in *Parksosaurus* (Upper Cretaceous), on the other hand, the cleft is apparently absent⁷. The distal end of the femur of *Hypsilophodon* (Fig. 1c and e) has practically no anterior intercondylar groove; this groove is, however, well developed in *Dryosaurus*, where the posterolateral condyle is thin, as it is in *Parksosaurus*⁷.

The posterior part of the right maxilla (Fig. 1d) was collected from the Lower Cretaceous Cedar Mountain Formation⁸ of south-eastern Utah. The teeth are very similar to some of those from *Iguanodon*⁹ (Fig. 1g) from Britain, and the Utah specimen is, therefore, probably referable to *Iguanodon* (D. Norman, personal communication). The thickly enamelled lateral surface of the crown has a prominent keel (Fig. 1d), whereas in *Tenontosaurus* only dentary teeth bear such a keel⁵. The presence of a deep vertical depression on the anterior and posterior edges of the root and base of the crown also serves to distinguish these teeth from those of *Tenontosaurus*⁵ and *Camptosaurus*¹. The distal end of a right femur from the same area as the maxilla has a very deep anterior intercondylar groove, as in *Iguanodon*³, again, this is in marked contrast to the shallow groove present on femora of *Tenontosaurus*⁵ and *Camptosaurus*¹. The distal end of a femur from the Dakota Sandstone of Nebraska¹⁰ can also be referred to *Iguanodon* on account of a very deep anterior intercondylar groove. The distal ends of the femora of certain hadrosaurs¹¹ are also similar to those of *Iguanodon*³, but hadrosaurs are not known to occur so early, the oldest known specimen from North America being late Cretaceous in age (Santonian, Mississippi)¹², though older records come from the Cenomanian of England¹³ and the Aptian-Albian of Mongolia¹⁴.

The North Atlantic started to open during the early Jurassic¹⁵ with a second stage of expansion, corresponding to the Bermuda discontinuity, probably ending sometime during the late Jurassic or early Cretaceous¹⁵. The presence of a 'transatlantic' land connection during the Middle Jurassic is clearly shown by the great similarity of the Upper Jurassic dinosaur faunas best known from the Morrison Formation of North America and the Tendaguru of Tanzania¹⁶⁻¹⁸. There are, however, no dinosaurian genera common to the Wealden Beds of Europe and either the Arundel Formation¹⁹ (Neocomian) of Maryland or the Cloverly Formation⁵. The specimens of *Hypsilophodon* and *Iguanodon* described here show that the presumed absence of these genera from North America reflect the lack of material from beds of appropriate age and/or ecology.

Reconstructions of the northern limit of the North Atlantic at about the time of the Jurassic-Cretaceous transition vary (Fig. 1a) but, taking into account epicontinental seas, the North Atlantic probably connected with the Arctic Ocean at that time^{20,21}. Epicontinental seas are, however, shallow and liable to retreat and advance locally, so they are not such an absolute barrier to terrestrial animals as are deep oceans. The presence of two characteristically European genera in North America indicates a northern land route between these two areas at about, or immediately before the Jurassic-Cretaceous transition. Future discoveries in the Neocomian of North America will probably increase the number of genera common to both areas. During the early Cretaceous the Mid-Continental Seaway of central

North America (Fig. 1a) had not extended far enough north to separate two distinct dinosaurian faunas in the northern hemisphere (Asiaamerica, Euramerica), as occurred in the Upper Cretaceous¹⁷.

The specimens discussed here will be fully described elsewhere²³.

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Membrane augmentation in freezing tolerance of plant cells

THE question of the special physiology and biochemistry of growth and adaptation of plants to low temperature has been raised¹ with reference to the significance of the increased unsaturation of fatty acids observed in such plants. Interest in the phenomenon of increased unsaturation with exposure to low temperatures, first documented by Hilditch², has received impetus from reports^{3,4} that membranes and membrane lipids of plants sensitive to chilling change from a liquid-crystalline to a gel structure when exposed to low temperatures, whereas those from plants resistant to chilling undergo no such phase change. The implication was that the less unsaturated membrane lipids of cells or organelles from plants which are sensitive to chilling were less fluid at lower temperatures and consequently lost normal function at such temperatures. Reports of increased fatty acid unsaturation in phospholipids of plant tissues^{5,6} which are tolerant of chilling lend credence to these views. The apparently reasonable extrapolation from these findings that has been made⁷⁻¹¹, and that was inferred by Moore¹, is that the resistance of plants to injury from actual freezing may also be derived from changes in unsaturation and fluidity of membrane lipids. This theory warrants closer examination. The two stresses of chilling and freezing are quite different; chilling resistance is an adaptation to low temperatures above freezing and principally involves the interaction between metabolic function and structure, whereas freezing resistance is tolerance to the dehydrative stresses of crystallisation of ice, and seems to be largely structural in nature^{12,13}. It is reasonable to expect, therefore, that the underlying chemistry of resistance to chilling and freezing would be different. Our findings indicate that this is the case.

Two conclusions can be drawn from our results (Table 1). First, in the transition of the living bark cells from the summer unhardy (LD₅₀ -10 °C) to the winter hardy (LD₅₀ > -196 °C)

Table 1 Fatty acid composition and PC:PE ratios of summer and winter living bark tissues of the black locust tree

Tissue	Phospholipid	Fatty acid composition (mol %)						Δ /mol*	PC:PE†
		16:0	18:0	18:1	18:2	18:3			
Summer (LD ₅₀ -10 °C)	Total	21.4	1.7	5.8	62.8	8.3		1.6	1.5
	PE	23.7	1.5	4.8	64.1	5.9		1.5	
	PC	15.7	2.2	6.7	63.1	12.2		1.7	
Winter (LD ₅₀ > -196 °C)	Total	19.8	0.4	2.3	69.7	7.7		1.6	1.6
	PE	23.2	0.4	1.8	69.1	5.5		1.6	
	PC	12.8	0.5	2.2	74.5	9.9		1.8	

Phospholipids extraction, separation and fatty acid determination were performed according to methods described previously¹¹.

* Δ /mol = $1.0 \times (\% \text{ monoene})/100 + 2.0 \times (\% \text{ diene})/100 + 3.0 \times (\% \text{ triene})/100$.

†By mol.

condition no significant change occurs in the fatty acid unsaturation of either of the two major phospholipids, phosphatidylcholine and phosphatidylethanolamine. These results extend our finding¹⁴ of a lack of change in unsaturation in both the total lipids and combined phospholipid fractions. Second, no significant shift occurs in the proportions of the two major phospholipid classes. These results agree with those of Yoshida and Sakai¹⁵ for the poplar tree. Changes in fluidity of membranes arising merely from increased unsaturation or from a shift in phospholipid classes cannot, therefore, be the factors responsible for the development of extreme freezing tolerance. In contrast to trees, herbaceous plants do undergo large increases in unsaturation during hardening^{9,10}, which must be a result of exposure to the low temperature regimes required for hardening since winter wheat cultivars of contrasting hardness exhibit the same degree of unsaturation¹⁶. It is also interesting that the relative unsaturation of tree cells in their hardest condition (LD₅₀ > -196 °C) is still less than that of cereals in their most tender state (LD₅₀ -2 °C).

So far the only change we have observed in the locust bark cells that is consistent with the development of extreme freezing tolerance is the augmentation of membranes. Table 2 demonstrates the quantitative augmentation of membrane components on a DNA or unit cell basis. The development of

Table 2 Augmentation of phospholipid and protein in insoluble homogenates of summer and winter black locust bark tissues.

Tissue	Temperature at		Phospholipid/DNA	Protein/DNA
	LD ₅₀			
Summer	-10 °C		4.7	14.0
Winter	> -196 °C		11.1	33.0

Insoluble homogenates were prepared as described in ref. 11. DNA was measured by estimation of adenine content after Schmidt-Thannhauser extraction and removal of RNA (J. S. and D. S., unpublished). LD₅₀ refers to the temperature at which 50% of the cells were killed. Values presented represented means of three replicates.

tolerance to freezing must, therefore, be associated directly with this increase in membranous material as we have also been able to produce, by starvation of these cells graded levels of resistance to freezing which correlate directly with graded concentrations of phospholipid¹³.

We suggest, therefore, that at least one of the mechanisms of resistance to freezing, and probably the major one in these trees, involves the augmentation of membranous material in their cells. The biochemical and biophysical mechanism by which membrane augmentation enables the cell to accommodate to the stresses of extreme freezing are being investigated further.

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Superoxide dismutase in photosynthetic organisms provides an evolutionary hypothesis

Two years ago it seemed that a unifying hypothesis accounting for the distribution of superoxide dismutase (SOD) in different organisms had been achieved. Thus the enzyme was present only in aerobes and aerotolerant anaerobes, but absent from obligate anaerobes¹; eukaryotes contained primarily the cuprein (Cu-Zn) type of dismutase (cyanide-sensitive) whereas in prokaryotes the enzyme had either manganese or iron at the catalytic site (cyanide-insensitive); finally, the presence of a manganodisutase in the matrix space of mitochondria was interpreted² in terms of a polyphyletic origin for eukaryotes with independent evolution of SOD in the prokaryotes (Mn-SOD) and proto-eukaryotes (Cu-Zn SOD) before the postulated symbiotic event.

Several subsequent findings, however, have justified the cautionary nature of Fridovich's closing remarks to his hypothesis³. Lavelle *et al.*⁴ found only a single, manganese-containing SOD in the luminous fungus *Pleurotus olearius*; Lindmark and Muller⁵ reported cyanide-insensitive SOD activity in two aerotolerant protozoa; Puget and Michelson⁶ isolated a bacteriocuprein from *Photobacterium leiognathii*; Hewitt and Morris⁷ detected SOD in a wide range of obligate anaerobes, and, finally, Asada *et al.*⁸ noted the absence of cyanide-sensitivity in SOD from all algal phyla studied.

We have made an electrophoretic survey of the occurrence of SOD in photosynthetic organisms, both prokaryotic (bacteria and blue-green algae) and eukaryotic (red and green algae) (Fig. 1), and have confirmed the individual results of Hewitt and Morris and of Asada *et al.* All the organisms we studied contain cyanide-insensitive SOD activity and, in the case of the green alga *Codium*, a part of this activity has been localised in the chloroplast stroma. This contrasts with the situation in higher plants in which, so far, only the Cu-Zn enzyme has been found⁹⁻¹¹. We wish to suggest a new scheme for the evolution of photosynthetic organisms, relating the findings on SOD to the con-

ditions in which the initial symbiotic events are thought to have taken place.

The bacteriostatic effect of oxygen on *Chlorobium* and *Chromatium* may result because SOD cannot prevent the inactivation of certain enzymes vital to their metabolism (for example, Fe-S proteins). We must presume either (1) that SOD evolved independently in obligate anaerobes or (2) that SOD evolved before oxygenic photosynthesis as a result of the Urey effect, that is the production of oxygen by ultraviolet photolysis of water¹². There is no general agreement on the level of atmospheric oxygen which this would have produced¹³, but, if the enzymes from *Chlorobium* and *Chromatium* are as similar as the gels suggest, SOD must have antedated the divergence of these lines. It might be argued that some of the multiple bands of activity (especially those of *Chromatium*) do not represent genuine isozymes and are artefacts of electrofocusing or staining; however, the three activities of aerobically-grown *Rps. spheroides* extracts can be separated by ion exchange chromatography and give consistent single bands on gels.

The following scheme for the evolution of eukaryotic algae is constructed from the premise of the symbiotic origin of subcellular organelles, although the experimental findings are not incompatible with alternative hypotheses¹⁴. The sequence of events would have been initiated by the acquisition, by a photosynthetic bacterium, of a water-splitting enzyme system linked to the photosynthetic electron transport chain, resulting in an oxygenic photosynthesis similar to that of present-day algae and plants. We have proposed that this enzyme, which contains manganese, may originally have had a dual role, functioning also, in a soluble form, as a SOD so that oxygen-evolving and oxygen radical detoxification mechanisms could have appeared simultaneously¹⁵. An immediate effect of this photosynthetic oxygen evolution would have been to force an aerobic respiratory metabolism on primitive nitrate-reducing bacteria¹⁶. Certain lines of these primitive aerobes may have evolved

mechanisms for phagocytosis and entered into a symbiotic relationship with primitive blue-green algae, resulting in a cyanome-like organism^{17,18}. This would have been to the benefit of both partners, the blue-green alga would have an environment of lower oxygen tension and the host cell would have a stable source of oxygen independent of the algal colonies.

A second symbiotic association, between a cyanome and an aerobic prokaryote would, when stabilised, produce an organism similar to a present day red alga. de Duve¹⁹ and Hall²⁰ have discussed how it may have benefited an aerobic host cell to enter such a relationship with another aerobe. Indeed, Hall makes the point that, if the host cell already contained enzymes duplicating many of the functions of the endosymbiont, then the latter may have become dependent on the host enzymes, thus explaining the nuclear encoding of mitochondrial proteins such as cytochrome *c* and SOD²¹.

Two important points must be made about the environment in which these events took place. Hall¹⁶ considers that a photosynthetic population large enough to produce a net increase in atmospheric oxygen could not have been achieved without the evolution of the environmentally less demanding eukaryotic algae. Thus the evolution of quite advanced forms of eukaryotes can be envisaged as taking place within a restricted, oxygen-rich environment, although the atmosphere at large remained anaerobic. Weyl²² has proposed that the tropical thermocline could have fulfilled this role, vertical diffusion of oxygen between the density layers of the ocean being greatly restricted. Second, it has been pointed out that, in a reducing atmosphere, copper would have been largely trapped as insoluble cuprous sulphides²³. Following the scheme outlined above, soluble copper would not have become available until after the evolution of eukaryotic algae, so that the earliest eukaryotes would not have contained Cu-Zn SOD.

The acquisition of Cu-Zn SOD by certain lines of eukaryotic organisms must now be considered. Given that closely related organisms survive whether or not they contain Cu-Zn SOD (for example, higher plants and algae, *Neurospora* and *Pleurotus*, *Photobacterium leiognathii* and other *Photobacterium* spp.), it can be inferred that there are no important functional differences between the types of SOD. We suggest that, while the potentialities of copper as a catalyst in biological systems were being explored (for example, in cytochrome oxidase and plastocyanin) a copper protein evolved in one or two lines which was, fortuitously, an efficient SOD and that this enzyme coexisted with, or in some cases replaced, the existing dismutase. Such a possibility is perhaps not unlikely in view of the SOD activity exhibited by quite simple copper-peptide chelates²⁴ and by galactose oxidase²⁵. The reactions between cupreins copper and various catechols²⁶ may be an indication of the now relict function for which the cupreins were originally evolved. Bacteriocuprein can be regarded as such a phenomenon occurring independently in a prokaryote—the only case so far detected because prokaryotes in general make less use of copper in enzymes²⁷.

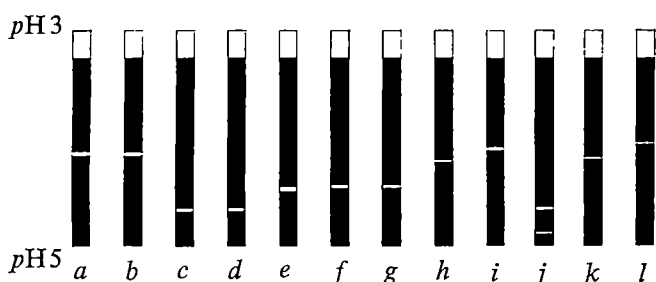
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Fig. 1 Polyacrylamide gel isoelectric focusing of SOD from photosynthetic organisms. SOD in soluble extracts of the organisms was partially purified by fractionation with ammonium sulphate (50–90% saturation) followed by dialysis. These crude extracts were assayed with or without 1mM KCN by the photochemical method of Beauchamp and Fridovich²⁸ (because of the instability of cyanide this was prepared on the day of use); then 2–10 U of SOD were applied per gel which was run²⁹ and stained²⁸ as described. See also ref. 11. Ampholytes were obtained from LKB Ltd. From left to right, the organisms are: *a*, *Chlorobium thiosulphatophilum* (green bacterium); *b*, *Chromatium D* (purple, sulphur bacterium); *c*, *Rhodospseudomonas spheroides* (purple, non-sulphur bacterium) anaerobically grown; *d*, *Rps. spheroides*, aerobically grown; *e*, *Nostoc muscorum* (blue-green alga); *f*, *Anabaena cylindrica* (blue-green alga); *g*, *A. flos-aquae* (blue-green alga); *h*, *Spirulina platensis* (blue-green alga); *i*, *Porphyridium cruentum* (red alga); *j*, *Scenedesmus obliquus* (green alga); *k*, *Codium fragile* (coenocytic green alga) chloroplast stromal extract; *l*, *C. fragile*, cytoplasmic extract. In the light of present knowledge, it has been assumed that insensitivity to inhibition by cyanide is a valid criterion of the absence of the cuprein-type of SOD from crude extracts. Nevertheless, until several of these enzymes have been characterised, the possibility of the existence of a class of cyanide-insensitive, copper-containing dismutases should be borne in mind.



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Cross-modal matching in the monkey after discrete temporal lobe lesions

ETTLINGER¹, reviewing the literature on cross-modal matching in primates, concluded that whereas man and chimpanzees seemed to be capable of such matching, the evidence concerning the monkey was ambiguous. Cowey and Weiskrantz², however, reported a method which resulted in a successful demonstration of a tactile-visual cross-modal match in normal rhesus monkeys. Their success seemed to result from the use of stimulus shapes made from powdered monkey diet, which the animal could eat. Such a training paradigm would be more natural—and therefore presumably easier—for the animal to master. In their experiment, monkeys were allowed to sample ten palatable and ten different unpalatable shapes (treated with quinine) in the dark; subsequently, the monkeys were given a choice between one palatable and one unpalatable shape, presented with the house light switched on. The monkeys chose the palatable shape significantly more often than the unpalatable, showing that a cross-modal match had occurred.

We have studied the effects of discrete temporal lobe lesions on cross-modal matching ability, using the Cowey and Weiskrantz method. Our subjects included three monkeys (two *Macaca mulatta* and one *Papio papio*) with foveal prestriate (FPS) lesions, two (one *M. mulatta* and one *P. papio*) with posterior inferotemporal (PIT) lesions and two monkeys (*M.*

mulatta) with anterior inferotemporal (AIT) lesions. These lesions are shown in Fig. 1. Each animal had an extensive history of testing on a visual delayed matching to sample task using colours, ellipses and curves as stimuli. These experiments showed that the FPS lesion produced no impairment, the PIT lesion resulted in animals with perceptual deficits and the AIT lesion produced an impairment that was significantly influenced by delay. In addition to these animals, two monkeys (*M. mulatta*) served as unoperated controls.

We used a standard Wisconsin general test apparatus (WGTA) and the transport cage which housed the monkeys was constructed so that shapes could not inadvertently become lodged within sight of the animal. Stimuli were prepared as follows: the positive shapes were made from Dixons powdered monkey diet (41-B) made into a dough like consistency with water (a small quantity of sugar was added to render them even more palatable), and then cut into various shapes with pastry cutters, and dried in a warm oven. Negative (unpalatable) shapes were made in a similar manner, except that no sugar was used, and a 5% aqueous solution of quinine dihydrochloride was used instead of water. The eighteen shapes made are illustrated in Fig. 2.

The monkeys were first trained to accept the experimental food in the dark in the WGTA. They were then presented with ten palatable and ten unpalatable shapes, in a counterbalanced design. For example, one monkey from a group received ten palatable crosses and ten unpalatable disks, while the other monkey received ten palatable disks and ten unpalatable crosses. All twenty shapes were presented simultaneously on a tray; the monkey was allowed to sample them at his leisure, typically taking about 10 min to do so. About 90% of the unpalatable shapes were discarded intact. The bottom of the transport cage was then covered with a cardboard sheet to prevent the animal from subsequently noticing any discarded stimuli, and the house light was switched on. For the next 20 s, the animal was shown one palatable and one unpalatable shape. Both these stimuli were manipulated by the experimenter so that the animal saw them from all possible angles. They were then placed side by side on a tray, and presented to the animal, who was permitted only one choice. This procedure was repeated daily, with a different pair of shapes being used on different days. These shapes were recombined after all nine pairs had been presented. In all, twenty choices per animal were recorded.

At the conclusion of the cross-modal study, all animals were given visual discrimination problems, using the palatable and

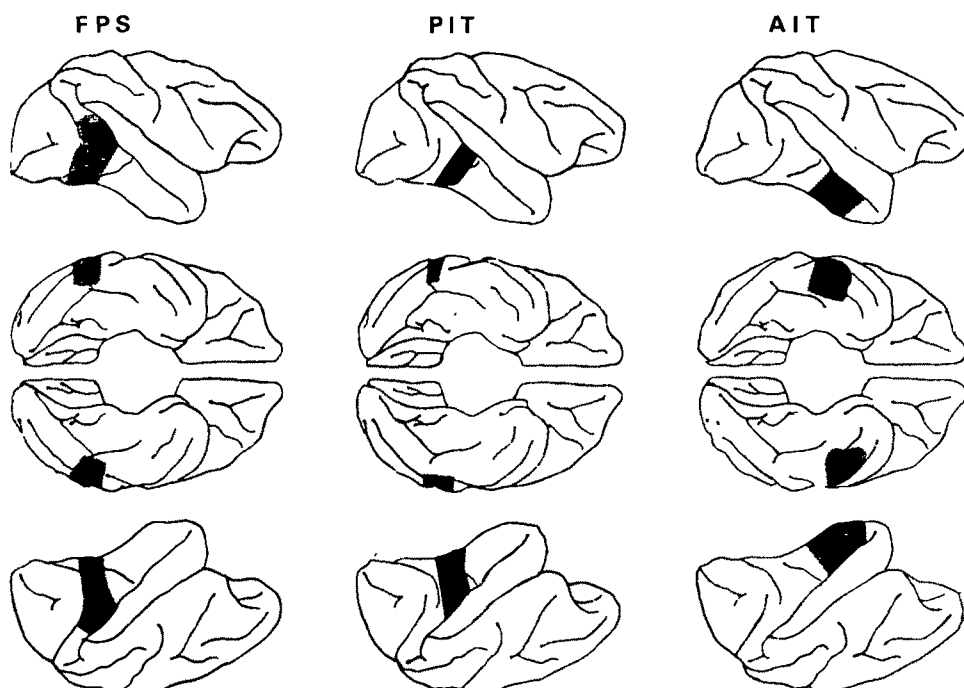


Fig. 1 Common (black) and maximum (stippled) extent of the lesions. For FPS $N = 3$, for PIT $N = 2$ and for AIT $N = 2$. The FPS lesion was designed to extend from the lower limits of the lunate sulcus to the superior temporal sulcus and from the lunate sulcus ventrally to the inferior occipital sulcus, thus including part of the foveal representation of Zeki's³ area V4. The AIT lesion extended anteriorly from the inferior occipital sulcus, with a lateral extent from the superior temporal sulcus to the ventral temporal lobe. The PIT plus AIT damage together constitute the traditional inferotemporal or TE lesion.

Table 1 Performance of individual subjects expressed as percentage correct on cross-modal matching and visual discrimination tasks

Animal and lesion	Cross-modal percentage corrects	P binomial two-tail test	Visual discrimination percentage corrects	P binomial two-tail test
Friend FPS	60		45	
Eunuch FPS	35	0.8	70	0.0358
Judge FPS	50		68	
Schizophrenic PIT	45	0.65	68	0.00014
Man PIT	60		78	
Fool AIT	75	0.0046	73	0.00014
Cockatoo AIT	75		73	
Jumper N	75	0.0114	73	0.00032
James N	70		76	

unpalatable shapes. Each animal had ten trials daily, using different pairs of stimulus shapes on different days, for a total of 4 d. This experiment was undertaken to see if the impairment in cross-modal matching could be attributed to a gross perceptual and/or mnemonic dysfunction within the visual modality.

Table 1 shows the results on cross-modal matching. The ability of normal monkeys to make a cross-modal match correctly, as determined by Cowey and Weiskrantz, was confirmed ($P = 0.0114$, binomial two-tailed). Both FPS and the PIT lesions impaired cross-modal matching ($P = 0.8$ and 0.65 respectively), whereas the AIT lesions did not ($P = 0.0046$).

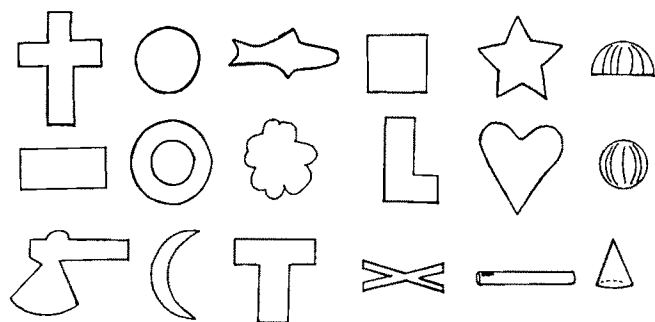


Fig. 2 Outlines of the eighteen shapes used, as viewed vertically. Their size can be judged from the first shape (cross), which was 5 cm along its short axis.

Statistical comparison indicated that the groups differed (information statistic⁴, $2\bar{I} = 11.728$, d.f. = 3, $P < 0.01$) and that this difference was between the AIT and the normal against the FPS and PIT groups ($2\bar{I} = 11.558$, d.f. = 1, $P < 0.001$). Furthermore, the differences between the AIT and the normals and between the FPS and PIT were not significant ($2\bar{I} = 0.170$, d.f. = 2, $P > 0.9$). On the right of Table 1 the overall percentages correct on the 40 trials on four discrimination tasks are presented, along with group significance levels, showing that none of the groups was impaired. A further analysis of the results on the visual discrimination tasks is presented in Table 2. An analysis by lesion group of the number of correct and incorrect choices on trials 2–6 on the four problems revealed no significant differences in visual discrimination performance (information statistic⁴, $2\bar{I} = 8.88$, d.f. = 15, $P > 0.8$).

The finding that the AIT group succeeded in matching whereas the PIT and the FPS animals failed was somewhat surprising, especially in view of the findings in the delayed matching to sample tasks described above. One plausible explanation stems from the findings of Iwai and Mishkin⁵, that AIT lesions result in a visual mnemonic deficit, although the more posterior lesions such as the PIT and FPS result in perceptual impairments. Furthermore, Iversen⁶ has shown that the visual memory deficit in inferotemporal lesioned monkeys is greatest when visual interference occurs. She found that inferotemporal monkeys kept in darkness after learning a visual discrimination problem were as good as normal monkeys in their ability to remember the task, whereas after new visual learning, retention was

greatly reduced. On this analysis, an AIT lesion would not impair cross-modal matching since much of the task is undertaken in the dark; and interference when the house light is switched on would be minimal, since the animal's attention is fixed on the relevant stimuli. In contrast, a PIT and a FPS lesion may impair the animal by causing a subtle visual perceptual breakdown. We do not think, however, that this is very likely. All our animals were equally good (with the exception of one FPS animal) at solving the visual discrimination problems, ruling out gross visual impairment. Since it is also known that temporal cortical lesions do not cause a tactile impairment (see ref. 7 for a review), the safest conclusion is that PIT and FPS lesions, in contrast to AIT lesions, result in a disruption of the mechanism that mediates a tactile-visual match. Further work is in progress to elucidate the common damage in the FPS and PIT lesions which underlies the reported impairment. It is worth noting that both lesions interrupt visual input to a multi-modal convergence area in the depths of the superior temporal sulcus^{8,9} and also includes partial damage to the sulcus itself. One of us (M.P.) is investigating the effects of bilateral lesions in the superior temporal sulcus, using cross modal matching and related tasks. Preliminary results suggest that there is a tactile visual cross-modal matching deficit.

Table 2 Performance matrix for the visual discrimination tasks

FPS (N = 3)	PIT (N = 2)	AIT (N = 2)	Control (N = 2)	
3 5 5 3 5	3 1 4 4 2	2 3 5 1 2	3 2 2 2 2	Incorrect
9 7 7 9 7	5 7 4 4 6	6 5 3 7 6	3 4 4 4 4	Correct
2 3 4 5 6	2 3 4 5 6	2 3 4 5 6	2 3 4 5 6	Trial No.

This analysis was performed only on trials 2–6, since the animal would of necessity perform at chance on the first trial, and after the sixth trial most problems had been learned. Thus, analysis based on trials 2–6 represents a conservative estimate of performance. The normal controls were tested on only three of the four visual problems.

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Spatial and temporal contrast sensitivity of striate cortical neurones

THE human visual system detects discontinuities in the spatial or temporal pattern of light falling on the retina; it is relatively poor at detecting steady or slowly changing patterns in either domain. These insensitivities to low rates of change in space and time are not independent: a gentle gradient of illumination can be made more visible by moving it, or modulating its brightness in time. Patterns of alternating light and dark bars with a sinusoidal luminance profile across the bars—sinusoidal gratings—are commonly used to study spatial interactions in the visual system. Some of the earliest workers to use these stimuli noticed that human sensitivity to steady sinusoidal gratings of low spatial frequency is poor, but is markedly enhanced if the grating is moved or modulated in time^{1,2}.

It has often been proposed that the lateral inhibitory mechanisms responsible for the system's relative insensitivity to low spatial frequencies operate with a slower time course than excitatory mechanisms, relatively reducing the effectiveness of lateral inhibition during the presentation of time-varying stimuli^{2–6}. Adaptation studies, however, suggest that temporal modulation does not change the spatial frequency tuning of individual spatial filters ('channels') in the human visual system^{7,8}; the increased detectability of temporally modulated low spatial frequency gratings could reflect the activity of a separate population of channels, sensitive to low spatial frequencies and relatively insensitive to steady stimuli^{8,9}.

The available neurophysiological evidence is equivocal. Lateral inhibition in the receptive fields of cat retinal ganglion cells is somewhat slower than excitation from their receptive field centres^{10,11}. But at the next stage of visual processing, the lateral geniculate nucleus, there is no difference between the time courses of excitatory and inhibitory mechanisms¹². We have examined the spatial frequency tuning of single neurones in cat striate cortex to establish whether their tuning is affected by the frequency of temporal modulation: does an increase in the temporal frequency of stimulation enhance a unit's sensitivity to low spatial frequencies?

Adult cats were prepared for electrophysiological recording using techniques described elsewhere¹³. Surgery was performed under short-acting barbiturate anaesthesia (Brietal); for recording, animals were artificially ventilated with a mixture of N₂O–O₂–CO₂ (78:20:2). The eyes were stabilised with an intravenous infusion of gallamine triethiodide (Flaxedil: 10–30 mg kg⁻¹ h⁻¹), coupled with bilateral cervical sympathectomy. Pupils were dilated with homatropine, and the corneas were protected with clear contact lenses. Artificial pupils (3 mm diameter) were placed directly in front of the eyes, and supplementary lenses were chosen to focus them on a screen 114 cm distant. The activity of single neurones was recorded with tungsten-in-glass micro-electrodes¹⁴ hydraulically advanced into the cortex through a sealed chamber over a small craniotomy and durotomy. Amplification and display of action potentials were conventional; a standard pulse triggered by each spike was fed to an audiometer, from which the experimenter judged the activity of the cell.

Receptive fields were initially mapped on a tangent screen

using flashed and moving stimuli, and classified according to the criteria of Hubel and Wiesel¹⁵. The dominant eye was chosen, and a cathode-ray oscilloscope, whose mean luminance was 150 cd m⁻², and which subtended 10° × 12.5° was centred on its receptive field; the other eye was covered.

Drifting sinusoidal gratings of different spatial and temporal frequencies, optimised for orientation and direction of movement, were generated on the oscilloscope face by a PDP-11/20 digital computer. The temporal frequency of a drifting grating is the number of cycles of the grating which pass any point on the screen in 1 s; its contrast is the difference between the luminances of its brightest and dimmest points divided by their sum. For any one cell, as many as forty different gratings were used—eight spatial frequencies, each drifting at five different temporal frequencies. The computer presented the gratings once each in random order, and the experimenter adjusted the contrast of each and turned it on and off under computer control until he judged that its introduction and withdrawal caused a liminal change in the activity of the cell. When a threshold had been estimated for each of the stimuli, their order was re-randomised and they were presented again. Four estimates of each threshold were obtained in this way; the standard error of their mean was normally between 0.1 and 0.2 log units.

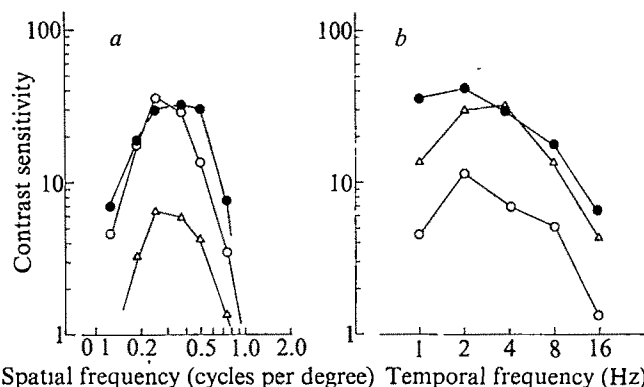


Fig. 1 Spatial and temporal contrast sensitivity functions of a simple cell. *a*, Spatial frequency tuning. The cell's contrast sensitivity as a function of the spatial frequency is shown for gratings drifting at three temporal frequencies. ○, 1 Hz; ●, 4 Hz; △, 16 Hz. *b*, Temporal frequency tuning. The contrast sensitivity as a function of temporal frequency is shown for gratings of three spatial frequencies. Contrast sensitivity is the reciprocal of the contrast at threshold, $(L_{max} + L_{min}) / (L_{max} - L_{min})$. ○, 0.13 cycles per degree; ●, 0.25 cycles per degree; △, 0.5 cycles per degree.

We examined the spatial frequency tuning of 48 units from 10 cats. Of these, 27 were simple, 18 complex and two non-oriented. We examined spatial frequency tuning as a function of temporal frequency in the manner described above in 26 of these units, 15 simple and 11 complex.

Figures 1 and 2 illustrate the results of two such experiments, performed on a simple cell and a complex cell recorded within 70 μm of each other. Figures 1*a* and 2*a* show that both cells were well-tuned for spatial frequency, and that the general form of their tuning curves was little affected by a 16-fold increase in temporal frequency. Neither the optimum spatial frequencies nor the relative low-frequency sensitivity was systematically changed. Although their overall sensitivity varied considerably with temporal frequency (Figs 1*b* and 2*b*) it is clear that the shapes of their temporal frequency tuning curves at different spatial frequencies were rather similar, apart from a slight tendency for the sensitivity to slow drift rates to be rather less at non-optimal spatial frequencies. This may reflect the fact that the responses of cortical cells to stimuli which are not optimal for the cell are more transient than those to optimal stimuli¹⁶.

The cells illustrated are typical: in no case did we observe

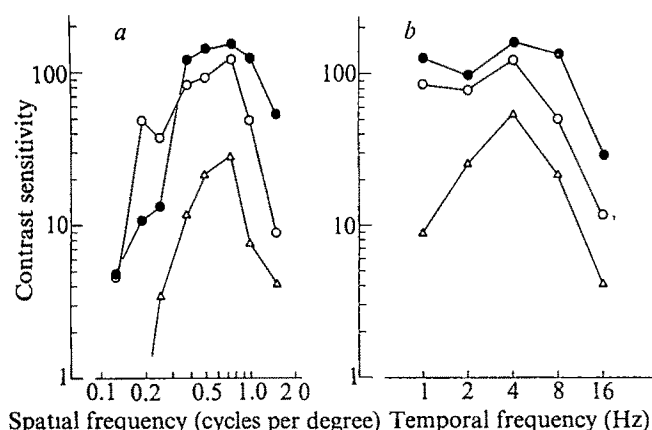


Fig. 2 Spatial and temporal contrast sensitivity functions of a complex cell. *a*, Spatial frequency tuning. \circ , 1 Hz; \bullet , 4 Hz; \triangle , 16 Hz. *b*, Temporal frequency tuning. Conventions as in Fig. 1. \circ , 0.38 cycles per degree; \bullet , 0.75 cycles per degree; \triangle , 1.5 cycles per degree.

any tendency for cells to become less well-tuned for spatial frequency at high temporal frequencies.

The results shown in Figs 1*b* and 2*b* also show that the sensitivity of the units to the velocity of stimulus movement depends on its spatial frequency. Expressed in terms of velocity in degrees per second (temporal frequency divided by spatial frequency), the cells' optima vary by a factor of eight to twelve over the range of spatial frequencies used. This contrasts with the situation when single moving bars are used as stimuli: in that case, the preferred velocity is independent of bar width¹³.

Our results may indicate that the effects of temporal modulation on human sensitivity to gratings of low spatial frequency cannot be explained by a change in the spatial frequency tuning characteristics of single neurones, unless neurones in the visual cortex of man are different from those in cat. Although we have not observed much variation among the temporal characteristics of striate neurones, it has been reported elsewhere¹⁴ that visual cortical neurones can be subdivided into a sustained group sensitive to high spatial frequencies and a transient group more sensitive to lower frequencies, which may represent the two populations of neurones required to explain the increase in low-frequency sensitivity produced by temporal modulation seen psychophysically.

It is also interesting that, since the sensitivity of units to the temporal frequency of drift does not depend on the spatial frequency of the stimulus, their selectivity in the domain of stimulus movement seems not to be for velocity but for temporal frequency, or the local rate of change in luminance with time.

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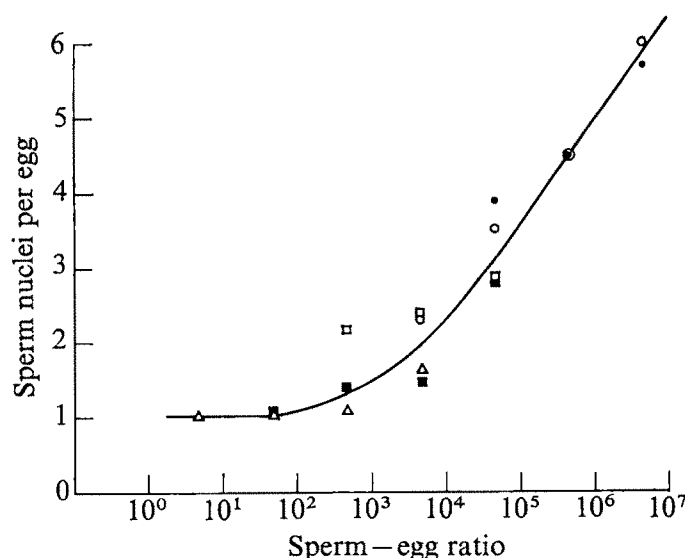
Absence of fast block to polyspermy in eggs of sea urchin *Strongylocentrotus purpuratus*

SEA urchin eggs remain monospermic during fertilisation by excluding the entry of all but one sperm. Two mechanisms have been proposed to account for this exclusion. The first is a rapid alteration of the egg surface, lowering its receptivity to sperm, which occurs within a few seconds of successful contact of the fertilising sperm; this is the so-called fast block¹. This block prevents or reduces further sperm entry for the next 30 s, until the time of the cortical reaction, which establishes the second and permanent block to polyspermy. This permanent or cortical block results from the release of cortical granule proteases, elevation of the fertilisation membrane, and subsequent detachment of sperm. The existence of a cortical block has been well established by a number of investigators²⁻⁴. Although the concept is intuitively compelling, the existence of a fast block has been questioned^{5,6}, and it is supported by only one series of experiments¹.

An opportunity arose to test the hypothesis of a fast block in sea urchin eggs when we found that by using high sperm-egg ratios, untreated *Strongylocentrotus purpuratus* eggs could be made highly polyspermic. In conditions in which many sperm enter each egg, we found no change in the frequency of sperm-egg fusions after fusion of the first sperm. We conclude that there is no fast block to polyspermy in *S. purpuratus*.

S. purpuratus gametes were shed after injection of 0.5 M KCl into the body cavity. Egg jelly was removed by swirling eggs in acid seawater (adjusted to pH 5.0 with 0.1 N HCl) for 2 min. The egg suspension was then adjusted to pH 8.0 with 1 M Tris-seawater pH 8.0, and washed extensively with filtered seawater. Suspensions of eggs were inseminated and incubated before

Fig. 1 Induction of polyspermy by increasing sperm-egg ratios. Samples (1 ml) of *S. purpuratus* eggs (0.002-2.0%) were inseminated with 0.2 ml of diluted sperm. Sperm-egg suspensions were incubated for 12.5 min at 16 °C before fixation in 95% ethanol-glacial acetic acid (3:1) for 18 h with three changes of solution. Eggs were cleared in 45% acetic acid and decondensed sperm nuclei observed by phase contrast microscopy. Sperm nuclei were counted in 40-50 randomly selected eggs. Final sperm densities: \bullet , 4×10^8 ; \circ , 4×10^8 ; \square , 4×10^7 ; \blacksquare , 4×10^6 ; \triangle , 4×10^5 sperm per ml.



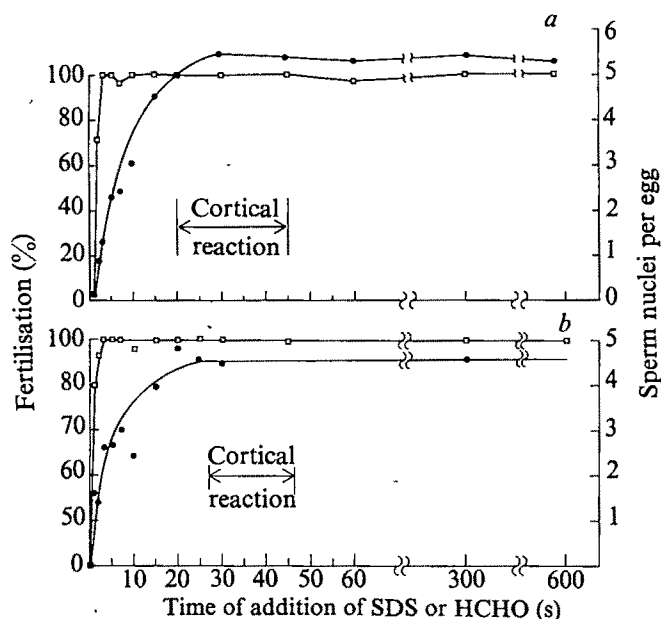


Fig. 2 Time course of sperm-egg fusions. *a*, samples (1 ml) of eggs were inseminated at time 0 by addition of 0.2 ml of sperm (8×10^7 per ml final). Eggs and sperm from the same sources were used throughout. Supernumerary sperm were killed by addition of 1 ml of 0.0075% SDS in seawater. This suspension was diluted with 4 ml of seawater 15 s later. Eggs were allowed to incubate before fixation as described in Fig. 1. Fertilisation was scored positive if a male pronucleus was visible within the egg. *b*, HCHO (0.037%) was substituted for SDS. All other procedures are the same as in *a*. \square , % fertilised; \bullet , number of sperm nuclei per egg (average value).

fixation. Eggs were cleared and decondensed sperm nuclei inside the eggs were counted using phase microscopy (Fig. 1).

We found that 100% polyspermy, with up to six sperm nuclei per egg, occurred in untreated *S. purpuratus* eggs fertilised at sperm-egg ratios greater than 10^4 (Fig. 1). This high level of polyspermy is normally achieved only by the use of polyspermy-inducing agents, such as nicotine or soy bean trypsin inhibitor. We believe our observations with untreated eggs result from the use of very high sperm-egg ratios, while most workers use ratios in the range 10^2 – 10^4 sperm per egg^{6,7}.

The observation that *S. purpuratus* eggs can become highly polyspermic suggests either (1) that at high enough sperm concentrations multiple sperm-egg fusions can occur before a fast block is established 2–3 s after insemination, or (2) that there is no complete fast block and sperm continue to fuse until the cortical block is established. To test these alternatives we determined when additional sperm were fusing with the egg in conditions which produced high levels of polyspermy. We added the spermicidal agent sodium dodecyl sulphate (SDS) or formaldehyde (HCHO) to a sperm-egg mixture at various times after insemination, to measure the number of fusion events which had occurred before their addition⁸. The addition of spermicidal agents at these times apparently prevents further sperm-egg fusion, but allows those sperm which have already fused to penetrate the egg and undergo nuclear decondensation. Accordingly we define sperm-egg fusion as an event which is a direct precursor of sperm penetration and nuclear decondensation, and which renders these subsequent processes insensitive to SDS or HCHO. Although we do not know the nature of the event which makes sperm penetration insensitive to SDS or HCHO, it is not simply binding of sperm to the egg surface, since hundreds of sperm bind at high sperm-egg ratios before the addition of spermicidal agents, but only a few subsequently enter the egg. It seems reasonable that the event which makes sperm penetration insensitive to SDS or HCHO is actual fusion of the sperm and egg plasma membranes.

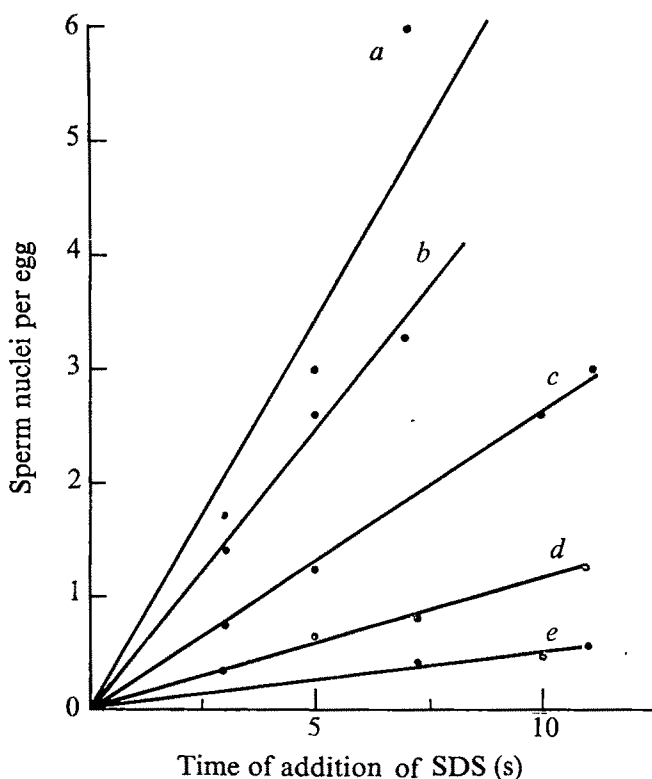
The timing of sperm-egg fusions was assayed by adding

0.0075% SDS or 0.037% HCHO to a sperm-egg suspension at various times after the gametes were mixed, and diluting 15 s later with the addition of 2 volumes of seawater. The eggs were fixed 12.5 min after insemination to count the decondensed sperm nuclei they contained. The concentration of spermicidal agent used was the minimum necessary to prevent fertilisation (that is, no elevation of a fertilisation membrane and no decondensed sperm nuclei) when added to eggs 1 s before sperm were added. The spermicidal agents were diluted routinely before this minimum effective concentration 15 s after their addition to limit their possible effect on the eggs. Although not done routinely, dilution could be performed at 1 s without altering the results, suggesting that SDS and HCHO are both fully effective within 1 s of their addition. These agents do not cause polyspermy by themselves, since the maximum level of polyspermy attained when they were present was no more than in control eggs not exposed to SDS or HCHO.

Figure 2 represents the results of a typical experiment to determine the time of occurrence of SDS or HCHO-sensitive sperm-egg fusions at sperm-to-egg ratios which produced polyspermy. A hundred per cent fertilisation, as judged by the subsequent appearance of at least one decondensed nucleus per egg, was achieved by 3–5 s after insemination; yet sperm-egg fusion continued for 25 s more. We conclude that fusion of the first sperm does not trigger an absolute block to the fusion of additional sperm. Our results support the concept of an absolute block to subsequent sperm entry which occurs at the time of the cortical reaction, since no additional fusion events occur after this time.

Although our results rule out a complete fast block to further sperm entry, there might have been a partial fast block (that is, a reduction in the rate of sperm-egg fusions after fusion of the first sperm) which the data in Fig. 2 do not rule out. If there were a partial fast block, the rate of sperm-egg fusions, as measured in a population of eggs, would decrease after 100%

Fig. 3 The time course of sperm-egg fusions, determined over a wide range of dilutions of a single sperm suspension. At the indicated times supernumerary sperm were killed by addition of SDS and the eggs were processed as under Fig. 2. Final sperm densities: *a*, 5×10^7 ; *b*, 2.5×10^7 ; *c*, 1.25×10^7 ; *d*, 5×10^6 ; *e*, 2.5×10^6 per ml.



fertilisation had been achieved, due to the lowered receptivity of the egg surface triggered by the first sperm. In the absence of any fast block, sperm-egg fusions would occur at the same rate before and after 100% fertilisation. The consistent result of experiments to test these alternatives critically is presented in Fig. 3. The rate of sperm-egg fusions was linear with time and proportional to the sperm-egg ratio. Moreover, in each case, the rate was identical before and after 100% fertilisation. We conclude that there is no change in egg receptivity to further sperm for at least 12 s after fusion of the first sperm, or no fast block.

Our conclusion that there is no fast block presents a paradox. As stated earlier, there are hundreds of sperm binding to the egg surface between the time of insemination and the complete cortical block. Without a fast block, how are all but a few of these supernumerary sperm excluded during this interval? We propose that the egg surface and surface coats act as filters to limit the fusion of these additional sperm. One such filter is the jelly coat of the egg, as proposed by Runnstrom and Manelli⁷. We have also found that in *S. purpuratus* eggs the intact jelly coat reduces the level of polyspermy obtained at moderate sperm-egg ratios below the level for eggs without a jelly coat. Even with the jelly layer removed, however, the number of bound sperm which can enter the egg is severely limited. The additional barrier may be a property of the egg's vitelline layer or the egg plasma membrane, which provide a limited number of effective sites or a time-delay barrier for sperm-egg fusion.

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Factors released from sea urchin eggs affect cyclic nucleotide metabolism in sperm

Cyclic nucleotide phosphodiesterase inhibitors and exogenous cyclic AMP or cyclic GMP can increase or maintain mammalian sperm motility, respiration rate and fructolytic rate¹⁻⁷. Sperm from various species have been shown to contain high activities of cyclic AMP-dependent protein kinase⁸⁻¹⁰, guanylate cyclase^{11,12}, cyclic nucleotide phosphodiesterase¹¹, phosphoprotein phosphatase¹³, and adenylate cyclase^{11,14}. These and other observations have led to speculation that cyclic nucleotides regulate or modulate sperm motility and metabolism¹⁵, and that this modulation may occur during the processes of capacitation or fertilisation¹⁶⁻¹⁷. The latter hypothesis requires that factors from the female or from the ovum itself alter sperm cyclic nucleotide levels. Until now there has been no demonstration of a naturally occurring factor of animal origin that can substantially change cyclic nucleotide levels in sperm¹⁵. The purpose of this study was to determine whether or not there are factors released from eggs that can alter sperm cyclic nucleotide levels. Gametes from sea urchins were used because both eggs and sperm can be obtained in large quantities, and

because factors released from sea urchin eggs have been known for some years to alter sea urchin sperm motility¹⁸⁻²⁰ and respiration^{20,21}.

Eggs and sperm were collected and washed as described previously²². Factors released from eggs (FRE) were obtained from the sea urchins, *Strongylocentrotus purpuratus* or *Lytechinus pictus*, by allowing eggs (10-20 mg wet weight ml⁻¹) to stand in a medium of simulated seawater²² for various times at 15-17 °C. The supernatant fluid above the eggs ('egg water') was collected by gentle centrifugation in a clinical centrifuge at 15-17 °C. The supernatant fluid containing FRE was added to incubation tubes containing artificial seawater with or without theophylline. To start the incubation (15-17 °C), 0.25 ml sperm suspended in artificial seawater (40-80 mg wet weight ml⁻¹) was added to each incubation tube. The incubations were terminated by addition of 1.0 ml 0.3 N perchloric acid containing traces of cyclic ³H-AMP and cyclic ³H-GMP to monitor recoveries. Cyclic AMP and cyclic GMP were purified and assayed as described elsewhere²³⁻²⁷.

Cyclic AMP concentrations in *S. purpuratus* sperm were increased approximately twofold by 1.5 mM theophylline after 1-min incubation (Table 1). FRE alone increased sperm cyclic AMP concentrations by about sevenfold, and the combination of FRE plus theophylline increased cyclic AMP concentration 100-fold. On the other hand, cyclic GMP in *S. purpuratus* sperm was decreased by about 40% by FRE. In contrast to their effects on cyclic AMP concentrations, theophylline plus FRE did not exert synergistic effects on cyclic GMP concentrations. Effects similar to those observed with FRE plus theophylline were observed with FRE plus 1-methyl-3-isobutylxanthine, another phosphodiesterase inhibitor.

Table 1 Effect of FRE from *S. purpuratus* on cyclic nucleotides in *S. purpuratus* sperm

FRE	Theophylline	Cyclic AMP (nmol per g wet weight sperm)	Cyclic GMP (nmol per g wet weight sperm)
None	None	2.5±0.7	0.76±0.12
None	1.5 mM	4.7±0.7	1.0±0.16
0.1 ml	None	16.6±2.0	0.46±0.04
0.1 ml	1.5 mM	267±26	1.13±0.16

Number of observations six; values represent mean ± s.e.m.

All sperm incubations were for 1 min at 15-17 °C.

Time zero cyclic AMP concentrations were 2.9±0.9 (*n* = 12) and those for cyclic GMP were 0.83±0.13 (*n* = 12) nmol per g wet weight. Time zero values were obtained by the addition of 0.3 N perchloric acid to incubation mixture before addition of the sperm.

Synergistic effects of FRE and theophylline on cyclic AMP content were also observed in sperm from *L. pictus*, although the changes were of smaller magnitude than in *S. purpuratus* sperm (Table 2). FRE from either *L. pictus* or *S. purpuratus* eggs in combination with theophylline increased cyclic AMP more than theophylline alone. Unlike the situation with *S. purpuratus* sperm, however, FRE alone did not increase cyclic AMP in *L. pictus* sperm (Table 2).

In addition to being increased by FRE and theophylline, cyclic AMP concentrations in *L. pictus* sperm were also increased by diluting the sperm in simulated seawater. Dilution of the sperm in the incubation mixture increased sperm cyclic AMP by about twofold by 1 min after dilution (Table 2). The increase was transient; by 10 min after dilution, concentrations were equal to or less than those at time zero. Such an effect of dilution was not seen with *S. purpuratus* sperm.

Because of the greater FRE-induced changes in cyclic AMP, *S. purpuratus* sperm were used in studies designed to partially characterise the cyclic AMP-elevating factor(s) present in FRE. The cyclic AMP response to increasing amounts of FRE in the presence of 1.5 mM theophylline seemed to be hyperbolic (data not shown). The same batch of FRE that was used to obtain the data in Table 1 caused a half-maximal increase in sperm cyclic AMP when about 10 µl were added to the incuba-

Table 2 Effects of FRE from *L. pictus* and of *S. purpuratus* on cyclic AMP in *L. pictus* sperm

	Theophylline (mM)	Cyclic AMP (nmol per g wet weight sperm)		
		1 min	10 min	30 min
No FRE	0	9.3±1.1	2.4±0.3	1.3±0.01
	0.15	13.4±1.1	3.4±0.3	—
	0.38	16.7±1.5	3.8±0.5	—
	1.5	21.1±1.6	5.1±1.4	3.8±0.6
<i>L. pictus</i> FRE (0.1 ml)	0	10.2±0.4	3.1±0.2	1.6±0.06
	0.15	22.4±1.7	4.9±0.3	—
	0.38	27.6±2.4	6.3±0.4	—
	1.5	40.5±3.7	9.6±0.8	5.8±0.2
<i>S. purpuratus</i> FRE (0.1 ml)	0	9.9±0.5	3.2±0.5	1.2±0.1
	0.15	17.3±0.9	4.0±0.2	—
	0.38	23.9±1.5	5.5±0.3	—
	1.5	35.0±2.2	8.3±0.4	5.5±0.8

Values represent mean ± s.e.m. of six observations at 1 and 10 min and of four observations at 30 min.

Time zero (see Table 1) cyclic AMP level in these sperm was 4.1 ± 2.1 ($n = 11$).

tion mixture. No increases were detected in cyclic GMP with up to 200 µl FRE in the presence of theophylline.

When a saturating amount of FRE was tested, there was some loss of cyclic AMP-elevating activity after dialysis, complete loss after ashing, but no significant loss after boiling FRE for 30 min. Two peaks of cyclic AMP-elevating activity were detected after filtration of 10 ml FRE on Sephadex G-50 columns (2.6 × 34 cm) equilibrated with simulated seawater. One of these peaks migrated in the void volume and was non-dialysable, whereas the other was retained by the column.

The mechanism of the FRE-induced elevation in cyclic AMP and reduction in cyclic GMP remains to be clarified. Direct stimulation of sperm adenylate cyclase by FRE in broken cell preparations has not been demonstrable, but extensive variations in assay conditions have not been examined. On the other hand, a potent inhibitor of sea urchin sperm guanylate cyclase in cell-free systems has been detected as a component of FRE

Table 3 Inhibition of sea urchin sperm guanylate cyclase by FRE

FRE (µl)	Guanylate cyclase activity (pmol cyclic GMP formed min ⁻¹)
0	744
5	527
10	409
25	299
50	263
100	211

Assay mixture contained 19.2 mM sodium azide, 12 mM theophylline, 0.45 mM GTP, 0.9 mM MnCl₂, 5 × 10⁶ c.p.m. ³H-GTP (5.6 Ci mmol⁻¹), the indicated amounts of FRE, 30 mM triethanolamine buffer at pH 7.8, and washed particles from 0.5 mg (wet weight) of sea urchin sperm²². The cyclic ³H-GMP formed during 15 min at 30 °C was determined as described elsewhere¹².

(Table 3). This material is stable to boiling, non-dialysable, and destroyed by ashing. The inhibitor is not adsorbed by charcoal and is not extracted from the simulated seawater by benzene or ether. Its inhibitory potency is much greater at low than at high sperm protein concentrations. Whether or not the inhibitor has any selectivity for guanylate cyclase over other enzymes, and whether or not it is the same as the non-dialysable substance(s) that increase cyclic AMP concentrations is not yet known.

These data constitute the first demonstration of alterations of sperm cyclic nucleotide concentrations by possible natural effectors. The potencies of the substance(s) that inhibit guanylate cyclase and increase cyclic AMP concentrations in sperm from sea urchins raise the possibility of their physiological importance in regulating sperm motility and metabolism. The possible release of similar factors from eggs of other species should be explored.

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Taxis to a conjugation-inducing substance in the ciliate *Blepharisma*

FOR conjugation of *Blepharisma intermedium* to take place interaction of soluble gamones with the cells is essential for union between complementary mating types I and II (refs 1 and 2). Type I cells excrete gamone 1 which transforms type II cells so that they can unite. Type II cells excrete gamone 2 which similarly transforms type I cells. Moreover, each gamone promotes the production of the other. Gamone 1, blepharismone, is a glycoprotein of molecular weight 20,000 (refs 3 and 4). Gamone 2, blepharismone, is calcium 3-(2'-formylamino-5'-hydroxybenzoyl) lactate⁵.

In a preliminary observation, in which a capillary containing gamone was placed in a cell suspension, type I cells were attracted by gamone 2. The observation has been confirmed here by the improved method using a microscope slide and coverslip (Fig. 1).

Clones A₁ (albino), N, D₃s and 4a of *B. intermedium* were used. A₁ and N were mating type I and D₃s and 4a were mating type II. The general techniques for the culture and handling of cells have been described elsewhere¹. Unless specified, cells were washed with and suspended in buffer¹ and starved for at least 1 d before use. Gamone 1 was purified as indicated³. Synthetic gamone 2 was supplied by Dr T. Tokoroyama, Osaka City University, and purified before use^{5,6}. One unit of gamone activity was defined as the smallest amount of gamone activity that can induce homotypic cell union in 500 cells suspended in 1 ml buffer¹. It corresponds to activities of 0.06 and 1.0 ng of purified gamones 1 and 2, respectively.

The taxis value was defined as described in Table 1 and the semi-quantitative measurement showed that type I cells were

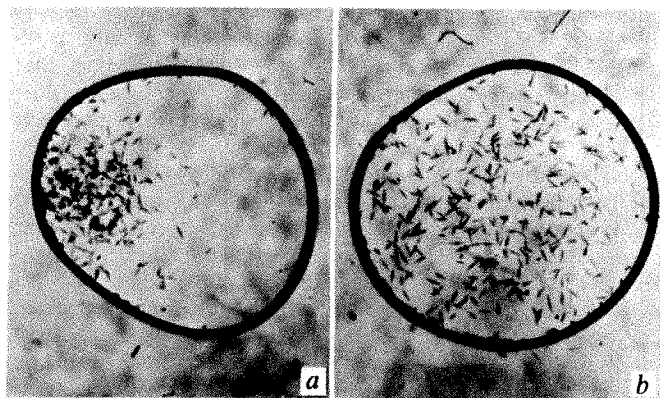


Fig. 1 Taxis of type I (A_1) cells to gamone 2. $10 \mu\text{l}$ of type I (A_1) cell suspension and $1 \mu\text{l}$ of cell-free fluid of the same cell suspension containing gamone 2 of $1,000 \text{ U ml}^{-1}$ (a) or none (b) were placed on a microscope slide and covered by a coverslip lifted 0.16 mm by spacers. The two drops were arranged so that, after covering, the smaller, satellite drop was situated very close to the left side of the larger, cell-containing drop. The two drops were then merged together by slightly moving the coverslip. Photographed after 30 min. Diameters of the disks were approximately 5 mm .

attracted by gamone 2 but not by gamone 1, while type II cells were attracted by neither (Table 1). The results were always reproduced in at least duplicate experiments.

Taxis values of type I (A_1) cells to different concentrations of gamone 2 are shown in Fig. 2. The threshold concentration of gamone 2 for the attraction may be estimated to be $10^{-6} \text{ U ml}^{-1}$ or $10^{-6} \text{ ng ml}^{-1}$. The sensitivity of type I cells to gamone 2 is, therefore, much higher in taxis than in the cell union.

Well-fed cells which are known not to respond to gamone 2 by cell union (H.H. and A.M., unpublished) were also not attracted by gamone 2. When such cells were washed, suspended in the buffer and observed during 9 h starvation, the taxis value suddenly increased between the third and the fourth hour reaching a maximum at the fourth hour (Fig. 3). Cell union also appeared after 4 h (Fig. 3). As the induction of cell union took 1 h in starved A_1 cells, the results indicate that the ability of cells to respond to gamone 2 by taxis and by cell union appears at nearly the same time, after 3 h of starvation.

The treatment of A_1 cells by cycloheximide ($20 \mu\text{g ml}^{-1}$, 2.5 h) did not change the ability of cells to be attracted by gamone 2. This treatment completely inhibits protein synthesis and the formation of cell union, which requires protein synthesis⁷ (H.H. and A.M., unpublished), suggesting that cell union and

Fig. 2 Taxis values of type I (A_1) cells to different concentrations of gamone 2.

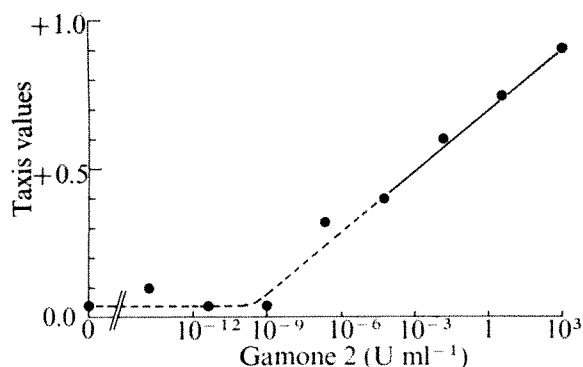


Table 1 Taxis values of mating types I and II of *B. intermedium* to their gamones

Attractants	Cells			
	Mating type I		Mating type II	
	A_1	N	4a	D_{3s}
Gamone 1	-0.03	0.00	0.00	0.06
Gamone 2	0.84	0.61	0.01	-0.03
Control (cell-free fluid)	-0.01	0.02	0.01	-0.01

Taxis values were obtained by the similar apparatus in Fig. 1, using gamones 1 and 2 of $4,000$ and $1,000 \text{ U ml}^{-1}$ respectively except the one marked by * in which gamone 1 was 130 U ml^{-1} . After 30 min from mergence of two drops, the cells were counted in each half of the drop parted evenly by a plane perpendicular to the line which passes through the centre of the cell-containing drop and the merging point of the two drops. The attraction of cells was expressed by the taxis value, $(a-b)/(a+b)$, where a and b were the number of cells in one half containing the merging point and the number of cells in the other half. Each value was the average of the values obtained by four experiments in which the relative position of the drops was changed by rotating the axis of the cell-containing drop, which was perpendicular to the surface of the coverslip, by 0° , 90° , 180° and 270° respectively.

taxis are independent. Whether gamone 2 receptors are different in these two responses is still to be determined.

Positive taxis of ciliates to the complementary mating type or its specific product has been reported only in *Tokophrya*. In this sessile ciliate, complementary mating types, if placed within a certain distance, orient and stretch toward each other (T. M. Sonneborn, personal communication, cited in ref. 2). In view of the obvious adaptive value of a sexual attractant

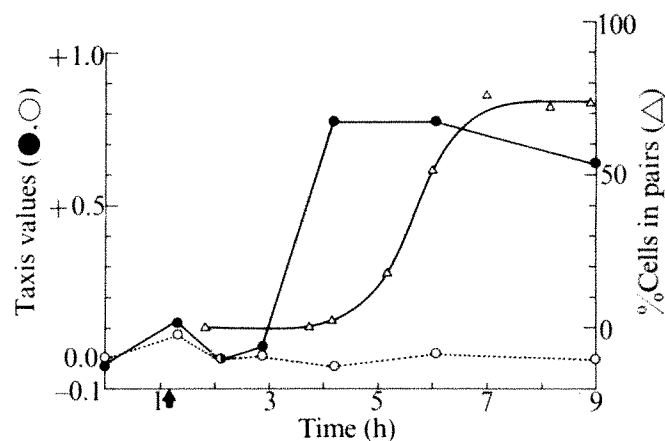


Fig. 3 Gamone taxis and gamone-induced cell union during starvation of well-fed type I (A_1) cells. Taxis values were measured on samples taken at different times during starvation. United cells were observed on a sample to which gamone 2 was added (800 U ml^{-1}) at the time indicated by the arrow. ●, Taxis to the cell-free fluid with gamone 2 ($1,000 \text{ U ml}^{-1}$); ○, taxis to the cell-free fluid without gamone 2; △, percentage of cell number in united cells.

and its general occurrence in eukaryotes, it is likely that more examples of mating type specific attraction will be found in ciliates.

The development of gamone taxis only in type I cells of *B. intermedium* may be compared with the fact that in many organisms only one of the two sexes attracts the other by a pheromone. The finding that type I cells respond to extremely low concentrations of gamone 2 demonstrates that ciliates have a highly sensitive sensory mechanism to specific molecules,

and thus introduces a new system of chemoreception amenable to molecular analysis.

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Biogenesis of chemotactic molecules by the arachidonate lipoxygenase system of platelets

BLOOD platelets contain two aggregation-activated enzyme systems that oxygenate arachidonic acid. One system is a cyclo-oxygenase that converts arachidonic acid into the biologically active endoperoxide prostaglandin G_2 (PGG_2), a precursor of classical prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$), as well as several non-prostanoid hydroxy fatty acids¹. The second aggregation-activated platelet enzyme is a lipoxygenase that transforms arachidonic acid into 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE)².

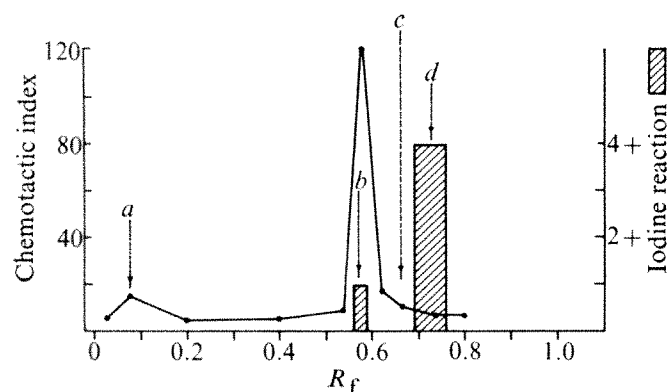


Fig. 1 TLC of the ether-extractable products obtained after incubation of 100 μ g of arachidonic acid with soluble platelet lipoxygenase (from 40 ml of human blood). Incubation was conducted in 0.1 M Tris-HCl/0.1 M K_2HPO_4 buffer (pH 8.2) at 30 °C for 90 min. Total reaction volume was 1 ml. Plates of silica gel 60 (Merck) (activated at 110 °C for 2 h) were developed to 15 cm with isopropyl ether-ethyl acetate-acetic acid (80:20:3 v/v). Chemotactic activity was assayed for one-fifth of each indicated fraction of the chromatogram and is expressed as a chemotactic index $CI = (C_a - C_b)/(C_c - C_b)$, where C_a , C_b and C_c are the average number of polymorphonuclear leukocyte indicator cells per $\times 430$ field attracted by the test agent (C_a), positive control: cell-free *Escherichia coli* growth medium (C_b), and buffer medium (C_c). Chemotaxis assays were performed in duplicate and five fields were counted and averaged for each assay. Chemotactic activity was primarily associated with the iodine-positive band at R_f 0.57 which was subsequently identified as HETE (see text). The concentration of HETE subjected to chemotaxis measurements was approximately 1 μ g ml^{-1} as estimated by GLC. Arrows indicate R_f values for lipid standards, a, PGE_1 , PGE_2 (R_f 0.07); b, ricinoleic acid (R_f 0.57); c, arachidonyl alcohol (R_f 0.66); d, arachidonic acid (R_f 0.72).

Although HETE is produced in significant quantities by aggregated platelets, no biological function for this compound has been reported. We now have evidence that HETE is a potent mediator of neutrophil chemotaxis.

We suggested³ that oxygenated arachidonate products may be the chemotactic mediators released by aggregated blood platelets⁴ because identical conditions are required for both arachidonate oxygenation and the generation of chemotactic activity^{2,4}. Since we had demonstrated that photo-oxidised arachidonic acid is chemotactic for neutrophils⁵, this hypothesis seemed even more tenable.

To test this hypothesis, chemotactic factor was generated by incubating the soluble lipoxygenase fraction² of human blood platelets with pure arachidonic acid. The chemotactic activity of the reaction mixture and its subsequent fractions was measured as before⁵, using non-autologous human polymorphonuclear leukocytes as indicator cells. After incubation, the products were acidified (pH 4) with 2 M citric acid and extracted into diethyl ether². All chemotactic activity was extracted into the ether phase, which was dried under nitrogen, resuspended in methanol, and subjected to thin-layer chromatography (TLC). All bands that could be visualised with the aid of 254-nm radiation, iodine vapour, and ninhydrin were removed from the chromatogram and tested for chemotactic activity.

As Fig. 1 shows, chemotactic activity was almost exclusively associated with an iodine-positive band at R_f 0.57. This material, evident as a dark absorption band under 254-nm illumination, exhibited no reaction with ninhydrin. The chemotactic band was clearly separated from authentic samples of PGE_1 , PGE_2 , arachidonyl alcohol, and arachidonic acid, but comigrated with ricinoleic acid. An iodine-positive band corresponding to unreacted arachidonic acid was also detected on the chromatogram. Another incubation, identical to the experiment described in Fig. 1 except for the addition of indomethacin (1 μ g ml^{-1}), gave a chemotactic profile almost identical to that shown in Fig. 1. This result indicated that the reactions studied were not due to the action of the platelet cyclo-oxygenase system which is inhibited by indomethacin at these concentrations¹. Other incubations which omitted the lipoxygenase or included lipoxygenase boiled for 30 min were not chemotactic and did not yield the R_f 0.57 band. These data suggested that platelet lipoxygenase was converting arachidonic acid into a chemotactic hydroxy fatty acid, probably HETE.

The presence of HETE was indicated by gas-liquid chromatography (GLC) (3% OV-225 on 120/100 Chromasorb WAW with column temperature 220 °C) of the active compound after catalytic reduction (H_2 -Pd on barium sulphate) and methylation (ethereal diazomethane). A single elution peak was observed which had the retention time of the methyl ester of a 20-carbon hydroxy fatty acid. The identity of the chemotactic material as HETE was confirmed by GLC-mass spectrometry (MS). A mass spectrometer (Finnigan 3300) fitted with a column of 1% OV-17 was programmed from 160–240 °C at 8 °C per min to obtain an electron impact fragmentation pattern of the reduced, methylated unknown. Prominent peaks at m/e 229, 200 and 197 were obtained which corresponded precisely to the reported mass spectrum of reduced, methylated HETE². The molecular ion ($M=342$) was absent.

To examine further the conditions required for the synthesis of chemotactic mediators, a series of incubations of platelets and platelet components with arachidonic acid was evaluated for chemotactic products using the same protocol as in Fig. 1. As Table 1 shows, chemotactic activity was always associated with the occurrence of an I_2 -positive TLC band at R_f 0.57 which had the GLC properties of a 20-carbon hydroxy fatty acid, indicating that HETE was the only chemotactic mediator elaborated by platelets.

Table 1 also indicates that both arachidonic acid and platelet-free serum function as prochemotactic substrates

Table 1 Reaction matrix for incubations conducted as described in Fig. 1

	Arachidonic acid (100 µg)	LPS (1 mg)	Platelet-free plasma (100 µl)
Whole platelets	+	+	+
Soluble lipoxigenase	+	—	+
Platelet acid extract	+	ND	+
Arachidonic acid, 100 µg	—	—	—
<i>S. typhosa</i> lipopolysaccharide 1 mg	—	—	—

The products of each incubation were worked up and assayed for chemotactic activity as outlined in Fig. 1 and the text. + indicates that HETE was the major chemotactic product as evidenced by TLC and GLC analysis. — denotes the absence of chemotactic products. Whole platelets², soluble lipoxigenase³, and platelet sulphuric acid extract⁶ were prepared from 40 ml of blood (0.1 mg platelet protein) according to the referenced procedures. Platelet-free plasma (PFP) was obtained by differential centrifugation of human blood⁴. Indicated quantities of arachidonic acid, LPS and PFP are for 1-ml incubation volumes. ND, not determined.

for any of the platelet fractions. This circumstance raises the possibility that arachidonic acid is the prochemotactic factor observed¹ in platelet-free plasma. Furthermore, the failure of *Salmonella typhosa* lipopolysaccharide (LPS) to generate chemotactic activity with soluble lipoxigenase suggests that LPS-generated chemotactic activity requires both lipoxigenase and arachidonic acid as found in platelet membranes, for example, whole platelets × LPS (Table 1).

HETE was also identified by TLC and GLC-MS as a component of the chemotactic material generated by ultraviolet photo-oxidation of arachidonic acid⁵. This observation indicates that non-enzymatic oxidation of arachidonic acid can generate a biological chemotactic mediator without intervention of either platelet lipoxigenase or the complement immune system. The status of complement components in chemotaxis is still an open question, especially in view of the observation that a crystalline preparation of the presumably chemotactic C5a was not chemotactic for neutrophils or macrophages⁶.

On the basis of our results, we propose that the platelet lipoxigenase system functions in a complementary fashion with the cyclo-oxygenase system to provide chemotactic, vasoactive, and platelet-aggregating factors during haemostasis. The likelihood of other biological activities for products of the platelet endoperoxidase and hydroperoxidase seems to be great and merits further investigation.

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Thymus-like lymph node in nude mice

PANTELOURIS has reported¹ that 1 out of 60 autopsied nude mice possessed a suspected normal thymus, but histological examination was not possible. Here, we report on five nude mice with distinct lobulated thymus-like organs in the thymus area, but which can be shown to be lymph nodes, rather than thymus.

Our nude mice were maintained by mating male *nu/nu* homozygotes with female *nu/+* heterozygotes (of outbred Swiss mouse background) at the veterinary Resources Branch, National Institutes of Health, Bethesda, Maryland. We have seen previously white lobulated lymphoid tissues, whose overall appearance is that of a 'normal thymus', in 5 out of 74 nude mice of 3–5 months of age. These mice were experimentally infected with either *Trichinella spiralis* or *Schistosoma mansoni* and were housed in specific pathogen-free conditions. Histologically these thymus-like tissues were lacking in Hassall's cor-

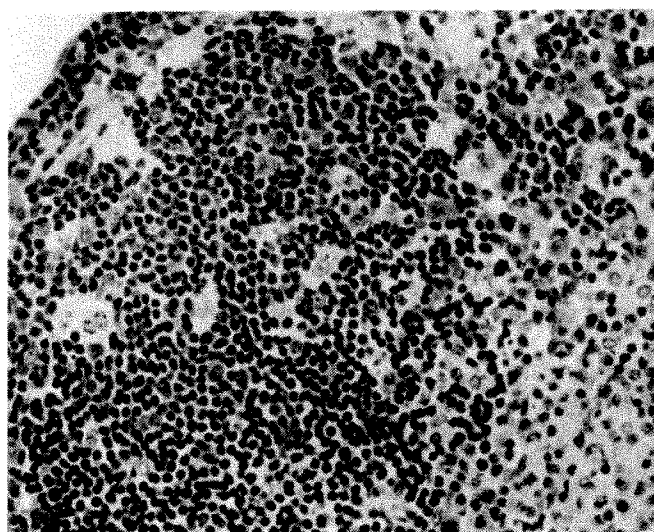


Fig. 1 No Hassall's corpuscles or thymic epithelial cells, but proliferative lymphocytes and reticuloendothelial cells were seen in this tissue (haematoxylin and eosin stain, ×300).

puscles and thymic epithelial cells. Active proliferation of lymphocytes, histocytes, and reticuloendothelial cells was evident.

In the cortex and medulla (Fig. 1) lymphoid follicles medullary sinuses and numerous plasma cells were also observed. These observations suggest that these tissues are active lymph nodes rather than normal thymus.

We have no real evidence to suggest that it is the pre-lymphoid thymic rudiment that is repopulated by the antigen-activated lymphocytes and histocytes, although this remains a possibility. These lymph nodes may be simply the anterior mediastinal or tracheobronchial lymph nodes that are greatly enlarged due to the proliferated immunolymphocytes and reticulendothelial cells, resulting from constant antigenic stimulation.

If the nude mice were born with a genuine thymus, one would expect to see the presence of functional T cells. Thus, spleen cells from the *Trichinella*-infected nude mice with suspected thymuses, infected and non-infected *nu/+* heterozygotes and typical nude mice without thymuses, were studied in response to phytohaemagglutinin (PHA), and *Salmonella typhimurium* lipopolysaccharide (LPS). These materials are generally accepted as mitogens for mouse T and B cells, respectively. The experiments were done in triplicate. No functional T cells were found, but B cells were demonstrated in the nude mice bearing suspected thymuses (Table 1). Since, however, crossing over

Table 1 Effect of infection on suspected thymus of nude mice

Mitogen	Dosage (μ g)	Trichinella-infected				Non-infected normal			
		c.p.m.	<i>nu/nu</i> * SI	c.p.m.	<i>nu/+</i> SI	c.p.m.	<i>nu/nu</i> SI	c.p.m.	<i>nu/+</i> SI
0	0	1,099		1,101		1,540		534	
PHA	8	692	0.64	10,129	9.20	1,405	0.92	12,327	20.08
LPS	20	35,441	32.40	48,870	44.30	77,313	50.20	2,214	41.6

*The nude mouse with suspected thymus
SI: stimulation index

might occur between the genes for athymia and hairlessness, if these are separated, we agree with Pantelouris that the experimenter should do gross necropsy and histology examinations to ensure that nude mice are really without a thymus.

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Unexpectedly rapid action of human interferon in physiological conditions

THE progression of many viral infections can be affected by the administration of exogenous interferon or the production of endogenous interferon^{1,2}, the concentration of which is greatest at the site of infection^{3,4}. Therapy with interferon generally requires large amounts^{5,6} presumably to allow for diffusion to the site. Model studies of this phenomenon *in vitro* have usually been carried out in the non-physiological conditions of relatively low concentrations of interferon and room temperature⁷⁻⁹. To understand better the factors that govern the local protective action of interferon, we have done model experiments in tissue culture to define the time and concentration variables under strict simulation of body temperature by carrying out all manipulations in a 37 °C water bath.

Duplicate HR203 human foreskin fibroblast cells were incubated in tubes 13 × 100 mm with prewarmed human leukocyte interferon (from the Antiviral Substances Program, NIAID)¹⁰, diluted in 0.5 ml Eagle's medium containing 2% foetal bovine serum. At various times the interferon was removed, and the cultures were washed four times with warm Earle's balanced salt solution and challenged with a Sindbis virus to cell input multiplicity of infection of 20:1. After 1 h for virus adsorption, cultures were washed three times and incubated for 18 h before culture fluids were assayed for viral haemagglutinin (HA).

Contrary to previous observations made in less physiological conditions⁷⁻⁹, a 30-min exposure of cells to interferon resulted in a high, although not maximal, interferon titre (Fig. 1a). Maximum titre was attained when the virus challenge was applied after 2–4 h of interferon treatment.

Shorter exposures to interferon were tried next. Figure 1b shows that a 1-min exposure resulted in almost the same interferon titre as that following 30 min of exposure. Similar results were obtained when the temperature was varied between

34.5 °C (temperature of upper respiratory tract) and 40 °C for a 5-min exposure.

To determine the effect of various concentrations of interferon on the level of resistance in the human cell cultures we applied either 10 U ml⁻¹, 30 U ml⁻¹ or 300 U ml⁻¹ of interferon to different cultures for various times before virus challenge. As Fig. 2 shows, the level of early antiviral activity was proportional to the concentration of interferon applied. Thus interferon at a concentration of more than 10 U ml⁻¹ was required to initiate the development of the early appearing resistance (8–16-fold inhibition) which is strong enough to be significant biologically.

Figure 2 suggests that resistance develops very slowly between 1 and 20 min of cellular reaction with interferon. Further study is required to understand the mechanism of this lag before the beginning of a second rise in cellular resistance, which leads to a higher and stable level of resistance⁸.

Several characteristics of the early developing antiviral activity suggest that it was induced by the interferon rather than by an extraneous substance. Its rapid development was species

Fig. 1 Development of interferon activity in HR203 cells challenged at various times after exposure to human interferon. The cultures were incubated with interferon dilutions for the times indicated, washed and challenged with virus. Interferon titres are expressed at the highest dilution which inhibited the virus yield threefold.

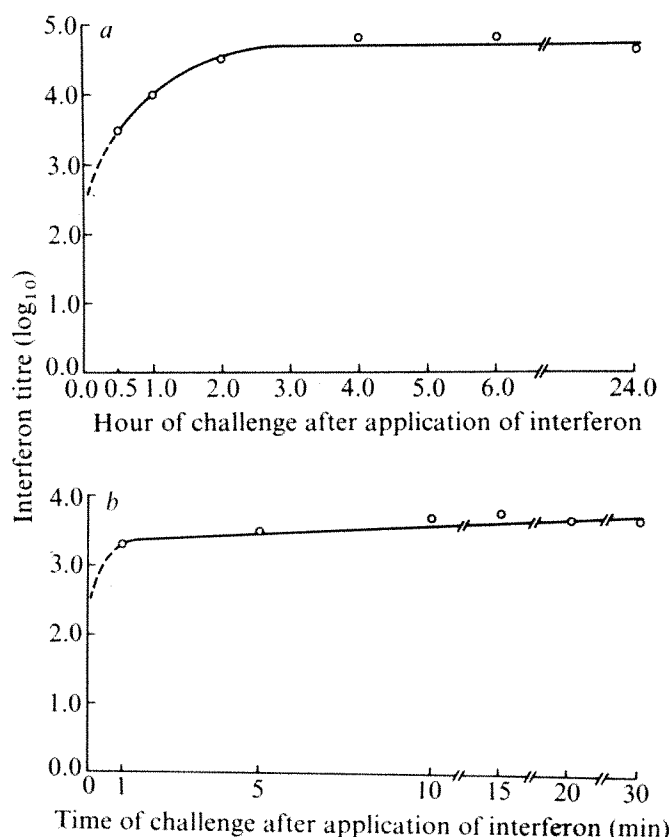
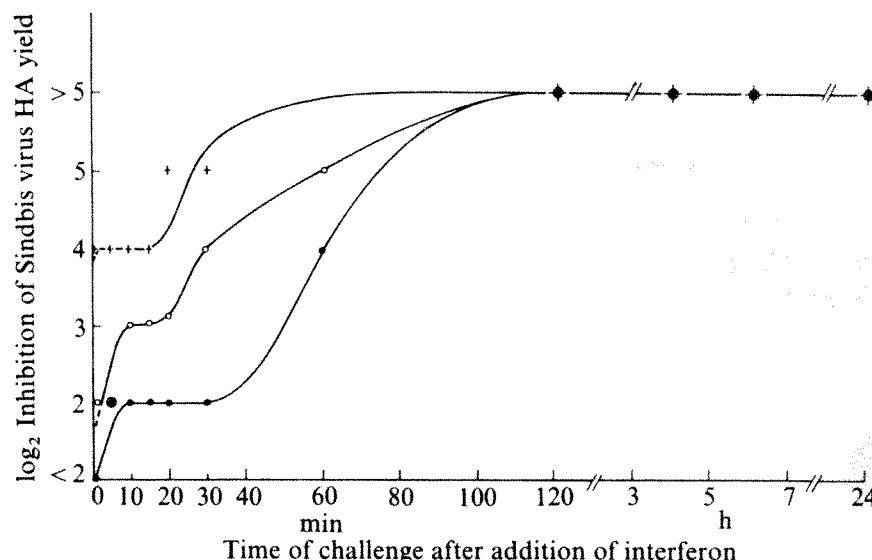


Fig. 2 Effect of interferon concentration on the level of rapidly developing resistance. Interferon was incubated with cultures for the times indicated before virus challenge. ●, 10 U ml⁻¹; ○, 30 U ml⁻¹; +, 100 U ml⁻¹.



specific, occurring in several strains of human diploid cells but not in mouse L cells. It was induced by both human leukocyte interferon and human fibroblast interferon. The same effect was observed in mouse L cells after a 5-min application of crude or semi-purified (10^7 U mg⁻¹ of protein) mouse interferon. The resistance manifested lack of viral specificity in that both vesicular stomatitis virus and the picornavirus, GD 7, were inhibited. Preincubation of the interferon with antibody to human interferon (obtained from Dr C. Anfinsen) or inhibition of RNA synthesis by actinomycin D prevented the development of early resistance.

The effect we have found is distinct from that reported when a brief exposure of cells in the cold to interferon, followed by prolonged incubation at 37 °C, was required for the development of antiviral activity¹¹⁻¹³. For example, prolonged incubation at 37 °C was not needed for the development of rapid resistance. Also, unlike the previous example, the reaction between interferon and cells at 37 °C seems to be due to direct induction of resistance. It is possible that in the cold the initial interaction of interferon with cells does not induce antiviral activity from the original binding site. The small amount of initially cell-bound interferon may simply serve as a reservoir of interferon during washing. Subsequently at 37 °C the interferon slowly elutes from the cells into the medium and then reacts with the cells to induce resistance¹⁴. In contrast, in the warm conditions we used, preliminary experiments involving antibody to interferon and repeated washings¹⁴ indicate that this type of cellular binding and subsequent elution are not required for development of rapid resistance.

Resistance to virus challenge after brief exposure to interferon does not necessarily mean that the resistance is established at the moment of virus challenge because several viruses, including Sindbis, may be inhibited when interferon is applied to cells as late as 2 h after virus^{15,16}. Nevertheless our preliminary experiments indicate that the messenger RNA for the resistance-inducing protein is transcribed as early as 30 min after a 5-min exposure of cells to 300 U interferon. Since translation of this mRNA is very rapid¹⁷ resistance probably develops after about 30 min.

To investigate whether concentrations of interferon sufficiently high for rapid effect *in vivo* occur, we estimated concentrations in the extracellular fluid around producing cells *in vivo*. We assumed that (1) a single infected cell releases its interferon into the surrounding intercellular space over a period of 6 h; (2) the half life of interferon in the extracellular fluid is similar to the rapid turnover phase in serum, that is, 10 min (ref. 3); (3) the volume of extracellular space surrounding a cell in a solid tissue varies from 12 to 120 μm^3 , and (4) that the interferon production *in vivo* may cover the same range as in cell

culture, that is 10–10,000 U ml⁻¹ in cell cultures or 0.00005–0.05 U per cell. Thus an estimated concentration of 3,000–30,000,000 U ml⁻¹ occurs in the intercellular space surrounding an interferon-producing cell *in vivo*. These levels are much more than the minimum of 10–30 U ml⁻¹ required to induce early resistance.

The biological significance of this rapid effect is clear. *In vivo*, the infected cell frequently produces both virus and interferon. A substantial lead of development of host resistance over the spreading virus would be provided by a rapid action of the interferon on surrounding cells since virus requires at least 1 h for adsorption, penetration and uncoating. This early defence would be less effective in conditions of minimal or late interferon production, low sensitivity of virus to interferon and a large volume of extracellular fluid.

The rapid action of interferon on cells has several additional implications. Is it analogous to the moderately rapid action of certain polypeptide hormones on cells^{2,18}? Also is it possible that the brief reaction induces a cellular event which, 30 min later, leads to derepression of the cistron encoded for the antiviral protein¹⁹? Study of the initial cellular event could yield information about the step(s) leading to derepression. Finally, can treatment of viral infections with interferon be improved by methods which deliver sufficient interferon to the site of infection to induce this rapid resistance?

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Immunity to Marek's disease induced by glutaraldehyde-treated cells of Marek's disease lymphoblastoid cell lines

MAREK'S disease (MD) is a common neoplastic disease of the domestic fowl caused by a herpesvirus (MDV). It is controlled in the field by vaccination with artificially attenuated strains of MDV, or more commonly with the antigenically related herpesvirus of turkeys (HVT)¹. The nature of the immune prophylaxis is not clear; protective immunity may be directed against MDV, against the development of tumours, or both. One way in which vaccination may exert its effect is by influencing the spread of MDV within the body². In contrast, infected birds may occasionally recover after showing signs of MD clinically, and in some birds MD lymphoproliferative lesions regress³, so it is likely that there is also an anti-tumour immune reaction. We have sought evidence for anti-tumour immunity in experimentally immunised birds.

MDV infection of chick kidney cells (CKCs) *in vitro* results in the expression of a number of nuclear, cytoplasmic and membrane antigens which can be visualised by immunofluorescence. Similar antigens occur on only a small proportion of MD lymphoma cells and MD lymphoblastoid line cells (about 10³ and 10 per million cells, respectively, in this study). The lymphoblastoid line cells and a variable (2–35%) proportion of MD lymphoma cells have other surface antigens which, because they occur only on cells which are thought to represent the malignant component of MD lymphomas, have been regarded as tumour specific antigens^{4,5}. The cells of lymphoblastoid lines from MD lymphomas contain the viral genome⁶, and the tumour specific antigens may be virally determined. Nevertheless it is possible to distinguish between viral antigens (as exemplified by MDV infection of CKCs) and tumour specific antigens (occurring only on non-productively infected lymphoblasts).

Lymphoblastoid line cells were used as a source of tumour specific antigens relatively uncontaminated with the known viral antigens, and CKCs infected *in vitro* with MDV were used as a source of these viral antigens. Glutaraldehyde is known to preserve or enhance the immunogenicity of antigenic structures⁷, and it was found to abolish the infectivity of MDV-infected cells in the conditions of this experiment. The induction

of tumour immunity in mice using glutaraldehyde-treated tumour cells has been reported for BALB/c tumours induced by methyl cholanthrene in BALB/c mice⁸. We have found that protection against MD can be achieved by immunisation with glutaraldehyde-treated lymphoblastoid lines as well as with similarly treated MDV-infected CKCs.

Groups of about 25 HPRS Rhode Island Red chickens were immunised (Table 1) with glutaraldehyde-inactivated tumour cells, infected lymphocytes, normal lymphocytes, lymphoblastoid cell lines or infected CKCs. A control group received no treatment, and another group was vaccinated with a standard MD vaccine (HVT). Immunised chickens were exposed to MDV at 55 d of age by mixing with birds which had been inoculated with MDV (HPRS 16 strain) 30 d previously, and which were actively shedding virulent virus. Mortality was observed until the birds were 230 d old. Birds which died from causes other than MD were eliminated from consideration. Diagnosis of MD as the cause of death was made on the basis of gross and histological visceral and neural lesions characteristic of MD⁹. The χ^2 test (with Yates's correction) was used to compare mortality in immunised groups with that of untreated birds (group F).

The results are shown in Fig. 1. As expected, birds which had been vaccinated with HVT or immunised with inactivated MDV infected CKCs were significantly protected against MD. Group G suffered a mortality of nine out of 22 ($\chi^2 = 5.54$) and group E of 5/19 ($\chi^2 = 9.96$) compared with group F (19/24). Group D, immunised with the lymphoblastoid cell lines, had a mortality of 10/23 ($\chi^2 = 4.91$). Mortality rates for the two individual cell lines were 6/13 ($\chi^2 = 2.82$) and 4/10 ($\chi^2 = 3.32$) for HPRS lines 1 and 2 respectively. The results for the two lines were consistent, and taken together the protective effect was significant at the 5% level.

The protective effect of inactivated preparations of productively infected cells, such as received by group E, is directed against virus replication or spread, as shown before^{10,11}. It is unlikely that the protection afforded by the treated lymphoblastoid cell lines was due to the viral antigens which are expressed on the small proportion of the cells which are productively infected, as the number of such cells received by group D was much less than was received by groups A and B (32- and 4-fold, respectively), which were not protected. The protective effect was probably due to the tumour specific antigens present on the cells of the lymphoblastoid cell lines. The failure of tumour cells and lymphocytes from infected chickens to protect may have been a dose effect. Malignant cells form only a minority of the cells of MD lymphomas, the remainder being inflammatory reactive cells^{4,5}. In contrast, almost 100% of the cells of MD lymphoblastoid lines carry tumour specific antigens^{4,5}. Alternatively, the tumour specific antigens present

Table 1 Immunisation schedules

Group	No. of birds*	Inoculum cell type	Age of recipients (d)		
			9	24	42
A	20	Tumour cells	21.4 × 10 ⁶ †	20.8 × 10 ⁶	80.6 × 10 ⁶
B	21	Infected lymphocytes	82.8 × 10 ⁶	76.0 × 10 ⁶	75.0 × 10 ⁶
C	24	Normal lymphocytes	94.8 × 10 ⁶	—	83.1 × 10 ⁶
D	23	Lymphoblastoid cell line cells	15.9 × 10 ⁶	—	26.2 × 10 ⁶
E	19	Infected CKCs	10 ^{3.2} PFU	—	10 ^{4.4} PFU
F	24	None	—	—	—
G	22	HVT (cell free)	10 ^{3.4} PFU	—	—

Tumour cells were freshly explanted cells of ovarian lymphomas from chickens experimentally infected with MDV (HPRS 16 strain). Infected lymphocytes were peripheral blood lymphocytes from the same birds. Normal lymphocytes were peripheral blood lymphocytes from unexposed chickens of the same age. Lymphoblastoid cell line cells were cells of HPRS lines 1 and 2, derived originally from MD lymphomas. Because of difficulties in obtaining large quantities of these cells, 13 birds were inoculated with HPRS line 1 and 10 with line 2. In view of the similarities between the two cell lines¹², these birds were treated as a single group. Infected CKCs were chicken kidney cells infected *in vitro* with HPRS 16 strain of MDV. All cells were assayed for infectivity on CKC monolayers before inactivation by incubation at 20 °C and at a concentration of 10⁸ ml⁻¹ for 15 min in 0.125% glutaraldehyde in phosphate buffered saline (PBS). After inactivation the cells were washed three times in PBS. HVT (cell free) was lyophilised herpesvirus of turkeys (strain FC 126). With the exception of group G, birds received the first inoculum in Freund's complete adjuvant, and subsequently without adjuvant.

* Birds dying of causes other than MD were removed from final group size.

† Quantities shown are doses per bird.

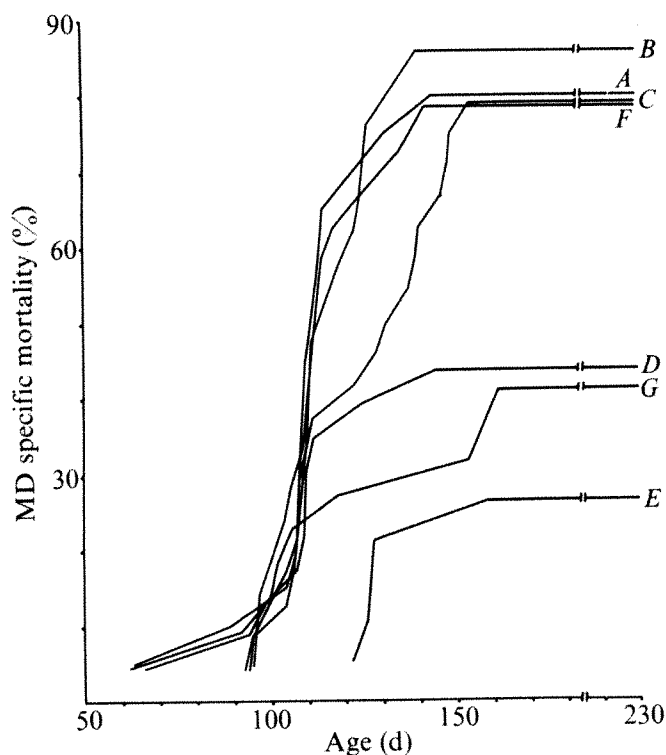


Fig. 1 Cumulative percentage of MD mortality of immunised and control chickens. Group treatments were as detailed in Table 1.

on lymphoma cells may differ from those on the lymphoblastoid cells of the cell lines, perhaps because of reaction with antibody or sensitised lymphocytes *in vivo*.

It has been suggested⁹ that resistance to MD proceeds by a two-step mechanism. The restriction of virulent virus infection brought about by vaccination may result in a much reduced incidence of malignant transformation of T lymphocytes, by reducing the probability of appropriate virus-cell interactions leading to neoplastic transformation. These transformed cells are the targets for the second step, the rejection of neoplastic cells. The immunosuppression caused by the multiplication of virus in the lymphoid organs may also exert an important influence on the outcome of infection. It is known from vaccination studies² and from experimental protection trials using inactivated virus preparations^{10,11} that immunity directed against virus specific antigens is protective. Vaccination, however, does not prevent superinfection with virulent strains of MDV, and the possibility of malignant clones arising still exists. These may be rejected by lymphocytes sensitised against tumour specific antigens. The results of our experiment show that resistance to MD may be induced with tumour specific antigens. The protective mechanism operating in birds which are resistant by virtue of vaccination, genetic composition or age may involve both these components. The nature of the tumour specific antigens and the role played by MDV in inducing their appearance remain unknown.

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Isolation of 14-nm virus-like particles from mouse brain infected with scrapie agent

SCRAPIE, a naturally occurring slow virus disease of sheep and goats, has been transmitted experimentally to the mouse, rat, gerbil, mink, hamster, vole and monkey¹. The agent has shown an unusual resistance to ultraviolet irradiation², nucleases³ and β -propiolactone⁴. The precise nature of the scrapie agent, however, has yet to be defined. It is not known whether it is a virus, some type of self-replicating cell membrane, or part of a membrane⁵. The viroid hypothesis of the scrapie agent of Diener⁶ has been discounted^{7,8}. Several different particles have been seen in thin sections of naturally and experimentally infected scrapie tissues⁹⁻¹¹. As repeated fluorocarbon extractions of mink tissues infected with Aleutian disease virus (ADV), which causes persistent infection of mink, have resulted in isolation of the virus^{12,13}, we thought it worthwhile to apply the same physico-chemical techniques in an attempt to isolate virus from mouse brains infected with scrapie agent.

The Chandler or Compton strain of mouse-adapted scrapie was obtained from Dr W. J. Hadlow¹⁴ (Rocky Mountain Laboratory, National Institute of Allergy and Infectious Diseases). Four hundred, 3-week-old female Swiss albino mice of the Animal Diseases Research Institute (Western) stock were inoculated intercerebrally with 0.03 ml of a 10^{-2} mouse brain suspension of the scrapie agent. Eighteen weeks after inoculation, clinical signs of scrapie appeared in many mice and the clinical disease progressed with time. During week 20 after infection some sick mice died. Starting in the week 20 after infection, brains were collected from severely affected mice or from dead mice. At 158 d after infection, all remaining mice were killed and their brains collected. Scrapie brains (132 g in total) were homogenised in 660 ml of saline using an omni-mixer at 15,000 r.p.m. for 15 min. Two parts of the homogenate were added to one part of Freon 113 (DuPont, trichlorotrifluoroethane, $\text{CCl}_2\text{F}-\text{CClF}_2$) and homogenised for 2 min at the same speed. After centrifugation of the Freon homogenate at 10,000g for 30 min, the supernatant was collected. The tissue phase was homogenised in saline and Freon extraction was repeated. Both supernatants were concentrated at 100,500g for 5 h. The ultracentrifuge supernatants were discarded and the pellets were collected. The pellets were suspended in 100 ml of saline, and Freon extraction and concentration of the supernatant were repeated. The final pellets were homogenised in 10 ml of 0.05 M Tris-HCl, pH 7.4, and labelled 'scrapie extract'. This scrapie extract contained protein (0.88 mg ml^{-1}) determined by the micro-Kjeldahl method. A 3.5-ml volume of CsCl solution (14.4 g of CsCl was added to 23.4 ml of 0.05 M Tris, pH 7.4) was put in a centrifuge tube and 1 ml of the scrapie extract was mixed with the top part of the CsCl solution. This was ultracentrifuged at 243,000g for 72 h using a Beckman swing bucket 50.1 Ti rotor. After ultracentrifugation, 10 equal fractions, each approximately 0.45 ml, were collected from the top of the tube using Pasteur pipettes. The pellet deposited in the bottom of the tube was also suspended in 0.45 ml of the Tris (fraction 11).

Two bands were visible in the CsCl gradient, one in fraction 5 and the other in fraction 7. Fractions 9, 10 and 11 were brownish in colour and appeared to contain ferritins. The buoyant

Table 1 Absorbance and electron microscopy of CsCl fractions of scrapie-infected mouse brain extract

Fraction No.	Absorbance		EM
	260 nm	280 nm	
1	0.255	0.191	No 14-nm particles
2	0.347	0.260	No 14-nm particles
3	2.561	2.376	No 14-nm particles
4	0.733	0.677	No 14-nm particles
5	2 × 2.644	2 × 2.597	No 14-nm particles
6	2.480	2.111	Some 14-nm particles
7	2.718	2.026	Numerous 14-nm particles
8	2.039	1.495	Some 14-nm particles and ferritin
9	4 × 2.034	4 × 1.178	Ferritin
10	8 × 2.530	8 × 1.309	Ferritin
11 (Pellets)	Not done		Ferritin

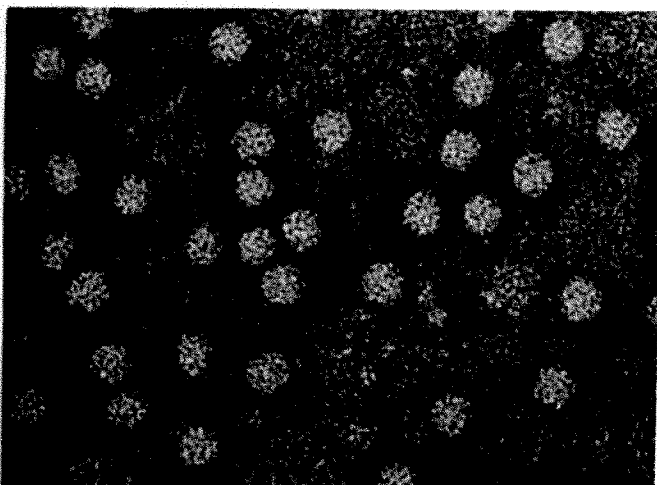
* Diluted with an equal amount of 0.05 M Tris.

densities of these two bands were determined from their refractive indices. The absorbance (A) of each fraction was read at 260 and 280 nm (Table 1). Then 4 ml Tris was added to each fraction and concentrated at 243,000g for 4 h. After ultracentrifugation, the supernatants were discarded and the pellets were resuspended in 0.4 ml of the Tris. For electron microscopy, a drop of each fraction was placed on to a Formvar membrane floating on cold distilled water and dialysed for 1 h. After this, one drop of the dialysed materials was placed on to a 400-mesh carbon coated grid, and allowed to deposit on the membrane of the grid for 3 min. The liquid on the grid was removed with a filter paper. Finally, the grid was stained with 2% potassium phosphotungstate, pH 7.2, and examined with an electron microscope (Hitachi model HU-12A).

Numerous virus-like particles, measuring about 14 nm in diameter, were observed in fraction 7 and lesser amounts were observed in fractions 6 and 8 (Fig. 1). The particles seemed to be spherical. Many were intact, but some seemed to be empty or incomplete. Fractions 8, 9, 10 and 11 contained ferritins¹³. No virus-like particles could be detected in fractions 1–5. These virus-like particles observed in the band in fraction 7 have a buoyant density 1.341–1.350 g cm⁻³, whereas the band observed in fraction 5 has a density of 1.296–1.305 g cm⁻³ in CsCl.

Similar extracts with 5-week-old normal mice brains from the same mouse colony yielded only one visible band in fraction 5, with a buoyant density of 1.296–1.305 g cm⁻³ in CsCl. No band was observed in fraction 7 from normal mice brain extracts. Electron microscopy of fraction 5 of the normal mice brain extracts did not reveal any particles. In the pool of fractions 6 to 8 some ferritin appeared, but no particles comparable with those seen in the scrapie infected mouse extracts.

Fig. 1 14-nm virus-like particles isolated from mouse brains infected with Chandler strain of scrapie agent ($\times 408,000$). Negatively stained with 2% potassium phosphotungstate, pH 7.2.



As known virus control, ADV was ultracentrifuged in the CsCl in the same run as a scrapie extract. The ADV was banded at a buoyant density of 1.405–1.416 g cm⁻³ which is typical of the parvovirus^{13,15}.

If the 14-nm particles isolated from scrapie-infected mouse brain are indeed a virus, they represent the smallest one ever reported. This size corresponds to the theoretical smallest size limit for a spherical virus¹⁶. It is not known whether these particles are indeed the scrapie agent. All the fractions shown in Table 1 are being tested by inoculation of mice to determine the amount of scrapie infectivity in each of them.

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Latent infection of the peripheral ANS with herpes simplex virus

In both experimentally infected animals^{1,2} and in asymptomatic human subjects^{3,4} herpes simplex virus (HSV) can establish a latent infection in the sensory ganglia of the nervous system. It is probably the periodic reactivation of virus within these ganglia that gives rise to recurrent herpetic eruptions on

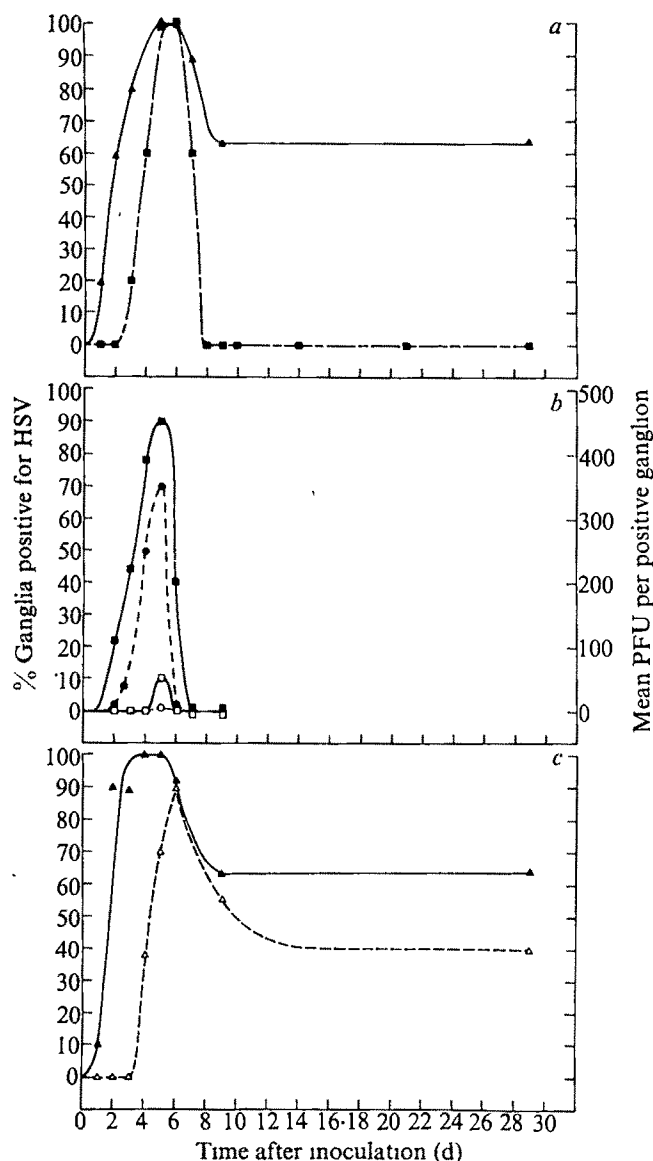


Fig. 1 Acute and latent HSV infection of the SCG after unilateral inoculation into the anterior chamber of the eye. *a*, Time course of SCG infection with HSV: the percentage of ipsilateral ganglia positive for virus by homogenisation (●) and explantation (▲) at various intervals after unilateral anterior chamber inoculation. *b*, Comparison of homogenates of ganglia ipsilateral and contralateral to the side of injection: homogenisation of ganglia at various times after unilateral anterior chamber injection presented both as the percentage of ipsilateral (●) and contralateral (○) ganglia positive for HSV and as the mean number of PFU per positive ipsilateral (●) and contralateral (○) ganglion. *c*, Comparison of explants of ganglia ipsilateral and contralateral to the side of injection: the percentage of ipsilateral (▲) and contralateral (△) ganglia positive for virus at various times after unilateral HSV injection of the anterior chamber. In all experiments the SCG of at least ten mice were assayed at each interval.

epithelial surfaces innervated by the infected ganglia. The ganglia of the peripheral autonomic nervous system seem to share a common embryogenesis with sensory ganglia⁵. Although it has been shown that acute infection of autonomic ganglia with HSV⁶ and the related herpesvirus, pseudorabies virus⁷⁻⁹, can occur, it has not been established that the peripheral autonomic nervous system (ANS) can support a latent infection with these or other viruses. We report here a murine model of latent infection of the superior cervical ganglion (SCG) of the sympathetic division of the ANS.

Four to six-week-old female BALB/c mice (Jackson Labora-

tories) were used. HSV (CHR-3 strain of type 1) was prepared and assayed on primary rabbit kidney (PRK) cells as described previously¹⁰. The SCG, which is situated near the bifurcation of the common carotid artery, was removed under a dissecting microscope in sterile conditions, washed three times in phosphate-buffered saline (PBS) containing antibiotics and assayed for the presence of HSV either by homogenisation or explantation; both carried out (with cocultivation on PRK monolayers) as described for sensory ganglia². Viral titres, expressed as the number of plaque-forming units (PFU) per ganglion, were determined on all homogenates positive for HSV.

The dilator muscle of the pupil is innervated by a rich network of adrenergic nerve terminals which have their cell bodies in the ipsilateral SCG¹¹. To expose these nerves to virus, 8×10^6 PFU of HSV suspended in 4 μ l were injected unilaterally into the anterior chamber of the eye using a 100- μ l syringe and repeating dispenser (Hamilton Co., Reno, Nevada); at various intervals thereafter the ipsilateral SCG was removed and assayed for virus. The data in Fig. 1*a* show that during the acute phase of infection, HSV was detected both in homogenates and explants of the SCG with maximum recovery of virus on days 5 and 6. The acute phase of infection ended at about day 8. Thereafter, homogenates were no longer positive, but HSV could still be recovered by explantation, indicating that the ganglia continued to harbour the virus.

To study the route of viral spread further, both ipsilateral and contralateral ganglia were assayed after unilateral inoculation of HSV into the anterior chamber of the eye. Comparison of bilateral homogenates (Fig. 1*b*) revealed that the amount of virus recovered from ganglia contralateral to the side of inoculation was less than 1% of that recovered from ipsilateral ganglia. Moreover, the occurrence of virus in explants of contralateral ganglia was delayed by 48–72 h compared with ipsilateral ganglia, in which virus could be detected as early as 24 h after inoculation (Fig. 1*c*). These studies indicate a direct, presumably neural, route of viral spread to the ipsilateral SCG. In contrast, spread to the contralateral SCG may involve an intermediary focus of infection (for example, pineal gland or brain parenchyma) although bilateral innervation of the iris or haematogenous viral spread cannot be ruled out.

Corneal inoculation with HSV (Table 1) led to a latent infection of the ipsilateral, but not contralateral, SCG (experiment A). The data from experiments B and C in Table 1 show that virus persisted in the autonomic ganglia for prolonged periods, lasting well over 1 yr. Since mice in experiments B and C were not tested earlier, the differences in the percentage of animals infected at 439 d compared with 114 d cannot be taken as indicating a decline in the prevalence of latent infection with time; assay of the trigeminal ganglia of animals in group C revealed a low prevalence of infection, suggesting a poor initial infection in this group.

The data in Table 2 show the effect of immunisation on the development of latent SCG infection. Mice were first immunised by intraperitoneal injection of HSV; 2 weeks later, when the mean serum neutralisation titre against HSV was 16, the animals were challenged with different doses of virus by anterior chamber inoculation. Mice were killed during the acute and chronic stages of infection, and the ipsilateral SCG was assayed for virus. High mortality and a latent infection of the SCG developed in unimmunised animals with a viral inoculum as low as 8×10^3 PFU. Immunisation prevented mortality and reduced by 80–100% the number of animals developing an infection of the SCG.

The events leading to HSV infection of the SCG can now be described. After anterior chamber inoculation, the virus probably contacts the abundant sympathetic fibres innervating the iris and passes by the neural route to the ipsilateral SCG. After corneal inoculation, either these same nerve fibres or those supplying the neighbouring blood vessels or lacrimal and nasopharyngeal glands serve as the point of viral entry leading to infection of the ipsilateral ganglion. The rapid development of HSV in the SCG supports the argument that virus can travel

Table 1 Latent infection of the superior cervical ganglion with HSV after corneal inoculation

Experiment	Side of assayed ganglion	Days after inoculation	Assay of ganglion by explantation Superior cervical	Trigeminal
A	Ipsilateral	4	11/19 (58%)	9/9 (100%)
	Ipsilateral	26	6/17 (35%)	7/9 (78%)
	Contralateral	26	0/16 (0%)	ND
B	Ipsilateral	114	17/39 (44%)	10/10 (100%)
C	Ipsilateral	439	7/42 (17%)	4/18 (22%)

Mice were inoculated with HSV by corneal scarification, and at different times thereafter the SCG and trigeminal ganglia were assayed for virus by explantation. Results are expressed as the ratio of the number of ganglia positive for HSV to the number of ganglia tested. Mice in experiment A were inoculated unilaterally using a virus pool containing 2×10^8 PFU ml⁻¹. Mice in experiments B and C were inoculated bilaterally with virus pools containing 1×10^8 and 1×10^7 PFU ml⁻¹ respectively. In these latter experiments the results of both the right and left ganglia were combined; thus the number of animals tested in experiments B and C is equal to half of the number of ganglia recorded.

ND, Not done.

Table 2 Effect of immunisation on the acquisition of latent superior cervical ganglion infection with HSV after inoculation into the anterior chamber of the eye

Group	Virus challenge (PFU)	Deaths	Days after inoculation		
			6 (Homogenates)	6 (Explants)	21 (Explants)
Unimmunised	8×10^6	11/30	8/10	10/10	15/19
	8×10^5	18/30	6/10	10/10	10/12
	8×10^4	8/30	5/10	8/10	8/21
	8×10^3	14/30	3/10	8/10	6/15
Immunised	8×10^6	0/15	0/7	1/8	3/15
	8×10^5	0/15	1/8	1/8	0/15
	8×10^4	0/15	0/9	0/8	0/15
	8×10^3	0/15	0/8	0/8	1/15
	None			0/15	0/15

Mice were divided into two groups; one group received a single 0.1 ml intraperitoneal injection of live HSV containing 2×10^6 PFU ml⁻¹, while the other group was not immunised. Two weeks later the animals were further subdivided and challenged with different doses of HSV by the anterior chamber route. Ipsilateral ganglia of mice killed at 6 d after challenge were assayed both by homogenisation and explantation, while ganglia obtained 21 d after challenge, were assayed by explantation alone. Results are expressed as the ratio of the number of ganglia positive for HSV to the number of ganglia tested. Deaths occurred between 7 and 10 d after anterior chamber inoculation and were tabulated at 21 d; the mice killed at 6 d were randomly selected and omitted from computation of mortality.

by direct retrograde axonal transport¹². Once the virus reaches the ganglion, productive viral replication ensues, as indicated by rising viral titres in homogenates. Fluorescent antibody studies reveal that 5 d after inoculation more than half of the neurones in the SCG contain viral antigens (unpublished). At 8 d after inoculation, the SCG homogenates become negative and HSV antigen can no longer be detected by immunofluorescence. Nonetheless, the infection persists, and virus can be recovered by explantation for at least 14 months.

In spite of differences in the organisation and functional roles of the autonomic and sensory ganglia, it seems that both types can support a latent HSV infection. Whether either type of ganglion actually becomes infected probably depends on whether their respective nerve endings are exposed to an adequate concentration of virus. Likewise, whether immunisation can prevent infection may well depend on the availability of anti-viral antibody and/or immune cells at the point where virus makes contact with nerve endings¹³. Our study shows that immunisation is quite effective in reducing the incidence of SCG infection after anterior chamber inoculation.

HSV infection of the SCG promises to be a useful animal model for the study of latent viral infection of the ANS. Moreover, this study raises the possibility that latent infection of autonomic ganglia with HSV or other viruses may well take place in man and could be responsible for periodic or prolonged dysfunction of target organs innervated by these ganglia.

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Interval coding of temperature by CNS neurones in thermoregulation

MODELS describing the central nervous control of thermoregulation^{1,2} are based at present on the discharge characteristics of temperature rate-sensitive neurones in the preoptic (PO) area and the hypothalamus. These neurones are believed to be involved in thermoregulation because they are anatomically located in an area affecting thermoregulatory control³⁻⁵ and respond to both local⁶⁻⁸ and peripheral⁹⁻¹¹ temperature change. Examination of the temporal distribution of interspike intervals from PO neurones in our experiments suggests that temperature rate-insensitive PO neurones also process thermoregulatory information.

We used adult New Zealand White rabbits, *Oryctolagus*

cuniculus in our experiments. Several probes were stereotactically implanted¹² in the brain: a thermocouple re-entrant tube in the left lateral PO, a thermode¹³ and a thermocouple re-entrant tube in the lateral border of the right PO and a moveable recording electrode array¹⁴ in the right medial PO equidistant from the thermode and re-entrant tube. The recording electrodes were prepared from 25 μ m diameter platinum-iridium wire. Extracellular potentials were first conditioned by a field effect transistor¹⁵, mounted on the head of the animals, and then amplified and filtered through routine bioelectric equipment. Discharges were typically from single neurones. Neural activity, as well as ambient, ear, brain and PO temperatures were recorded on magnetic tape for subsequent analysis. Neural activity was analysed by a PDP 8-I laboratory computer and represented in the form of mean firing rate and interspike interval (ISI) histograms. The ISI histogram is a plot of the distribution of time intervals between successive neural discharges.

After surgery, the rabbits were allowed to recover for at least 1 week. During experimental sessions, they were placed in a controlled ambient temperature chamber and were not anaesthetised or restrained. After obtaining a unit with a suitable signal-to-noise ratio, control unit activity was recorded for comparison with data collected at hot or cool clamped PO and ambient temperatures.

The experimental ISI histograms were compared statistically with their respective control histogram using χ^2 analysis and the F test. In each case, the experimental values were significantly different ($P < 0.01$) from the control; also, histograms generated at normal PO temperatures after each episode of heating or cooling did not differ significantly from their control histogram ($P > 0.05$).

Figure 1 shows ISI histograms for data from rate-insensitive neurone 2-E7 at an ambient temperature of 22 °C. Although the firing rate did not change significantly ($Q_{10}=1.02$), there was a significant change in the distribution of intervals during PO cooling. Clamping the PO temperature at 35.8 °C resulted in a bimodal distribution of intervals.

The ISI histograms in Fig. 2 indicate that some rate-insensitive neurones respond to both local cooling and heating with a change in interval distribution. These data from rate-insensitive neurone 11-E2 were obtained at 24 °C ambient temperature. At normal PO temperature, the

Fig. 1 Comparison of ISI histograms for rate-insensitive neurone 2-E7. *a*, Control; *b*, preoptic cooling. Each histogram represents 50 s of neural activity. The total number of occurrences of each interval width is expressed on the ordinate. Resolution of the interval width is 2 ms. t_{PO} , Preoptic temperature; t_A , ambient temperature; FR, firing rate (impulses $s^{-1} \pm s.e$)

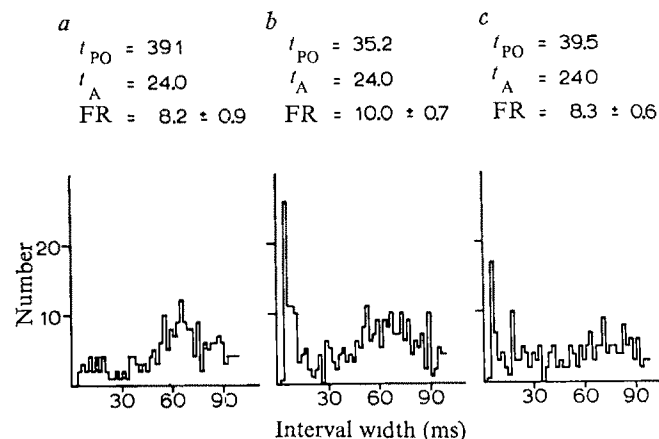
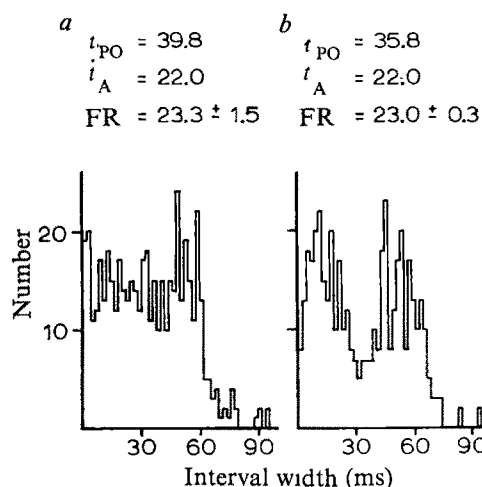


Fig. 2 Effects of preoptic cooling and heating on the interval distribution of rate-insensitive neurone 11-E2. *a*, Control; *b*, preoptic cooling; *c*, preoptic heating. Histogram details and definitions as in Fig. 1.

intervals were distributed unimodally, but during PO cooling or heating ($Q_{10}=0.6$ and 1.4 respectively), the intervals were distributed bimodally. In each case, the bimodal distribution resulted from the increased frequency of short intervals.

Since integrative thermoregulatory neurones receive information concerning external as well as internal temperature conditions, the ISI histograms were examined during ambient thermal stimulation. Figure 3 shows data from rate-insensitive neurone 17-L3 ($Q_{10}=0.9$). The interval distribution by this neurone is similarly by either PO or ambient thermal stimulation. The resulting bimodal distribution resembles that in Fig. 2 and was again a result of the increased frequency of short intervals. This type of neurone integrates thermal inputs from central and peripheral loci as expressed by its ISI distribution.

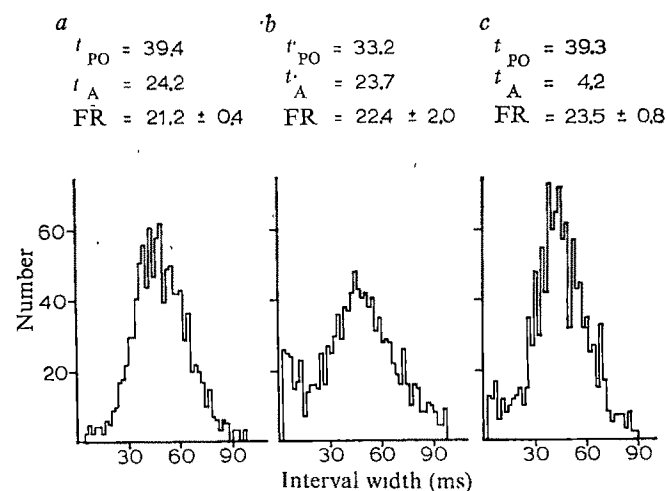


Fig. 3 Effects of ambient thermal stimulation on the interval distribution of rate-insensitive neurone 17-L3. *a*, Control; *b*, preoptic cooling; *c*, ambient cooling. Histogram details and definitions as in Fig. 1.

Models of the thermoregulatory system should include consideration of the importance of the ISI as a means of coding thermal information. In general the ISI histograms for these and other PO rate-insensitive neurones we have studied change significantly during thermal stimulation. Earlier studies^{16,17} indicated that only a small fraction (30%) of neurones in the PO are thermosensitive and considered to be involved with temperature regulation, however, firing rate was the only parameter used to determine

thermosensitivity and in addition, they dealt with anaesthetised animals. Consequently, our study may not conform to earlier results because our animals were capable of thermoregulation and not anaesthetised.

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Evoked transmitter release increased by inorganic mercury at frog neuromuscular junction

It is well documented that calcium ions have an important role in the release of transmitter substances from nerve terminals^{1,2}. There is, however, little information about the interactions of calcium with other chemical moieties within the nerve terminal which presumably are required to bring about transmitter release. Kosower and Werman³ postulated that calcium ions interact with two sulphhydryl groups to form a calcium dithiolate complex. The formation of such a complex results in a decrease in the sulphur-sulphur distance which is presumed to produce a microcontraction of the presynaptic membrane, thereby allowing for the quantal release of transmitter into the synaptic cleft. We show here that low concentrations of mercury ions (1 μ M or less) increase the amount of transmitter released from motor nerve terminals. Since it is well known that mercury ions dissociating from inorganic mercury (HgCl_2) form disulphide bridges in the presence of sulphhydryl groups, we interpret our observations as being consistent with Kosower and Werman's hypothesis.

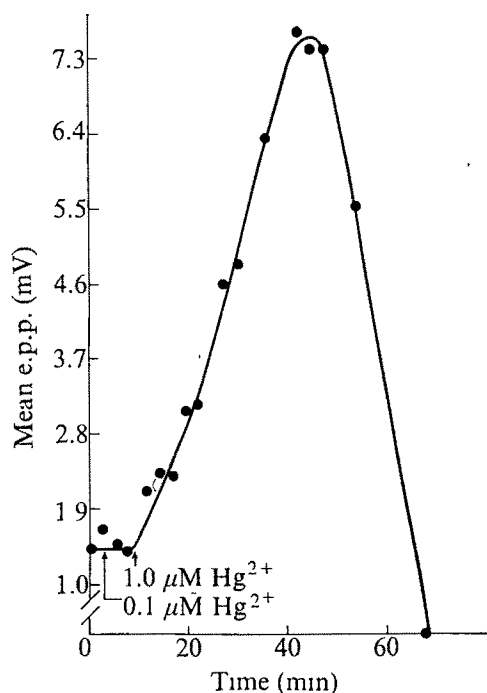
Experiments were performed *in vitro* on frog (*Rana pipiens*) sciatic nerve-sartorius muscle preparations maintained in a suitable chamber. Superficial neuromuscular junctions were viewed through a compound microscope with a total magnification of $\times 400$. Subthreshold endplate potentials (e.p.p.s) were produced by bathing the preparations in high magnesium/low calcium Ringer solution. The composition of the control Ringer solution was: 111 mM NaCl, 2.5 mM KCl, 0.36 mM CaCl_2 , 1.1 mM MgCl_2 , 4 mM Tris-maleate. Micromolar amounts of HgCl_2 were added to this Ringer solution to test for both presynaptic and postsynaptic effects of mercury ions. All Ringer solutions were maintained at pH 6.9 and 15 °C. Microelectrodes filled with 1 M potassium citrate were used for intracellular voltage measurements. A signal averager (Computer of Average Transients 1000) was used to record the mean e.p.p. To determine whether changes in the e.p.p. were

due to a direct effect of mercury ions on the postsynaptic receptors, these receptors were activated directly through the iontophoresis of acetylcholine (ACh) from a micropipette rather than indirectly by nerve stimulation, which results in the neural release of ACh.

Figure 1 shows that mercury had an intense potentiating effect on the e.p.p. amplitude. In this experiment, the mean e.p.p. amplitude was recorded during prolonged exposure of the preparation to 1.0 μ M Hg^{2+} . The e.p.p. increased from a control level of 1.4 mV to a value near 7.3 mV within 30–40 min of the beginning of the mercury exposure. The addition of 0.1 μ M Hg^{2+} a few minutes before the addition of the 1.0 μ M Hg^{2+} resulted in no detectable change in the e.p.p. amplitude. The percentage increase from control after addition of 1.0 μ M Hg^{2+} was 420%. The e.p.p. amplitude did not remain steady at this value of 7.3 mV, but it soon began to fall, eventually to zero. The increase in e.p.p. amplitude brought about by Hg^{2+} is very similar to the effect of diamide on the e.p.p. observed by Werman *et al.*⁴. These investigators suggest that diamide increases the e.p.p. amplitude by the formation of disulphide bonds in the presynaptic nerve terminal, which, according to their hypothesis, results in an increased quantal release of transmitter.

The potentiating effect of mercury ions on the e.p.p. occurs when mercury is present in low concentrations—either 1.0 μ M or less. In one experiment, 0.01 μ M mercury increased the e.p.p. by 33%; in another, 0.1 μ M mercury increased the e.p.p. by 26%. In the latter experiment, washing the preparation with mercury-free Ringer solution resulted in only a slight fall of the e.p.p. towards the control

Fig. 1 Effect of the e.p.p. of a prolonged exposure to 1.0 μ M HgCl_2 . The amplitude of the mean e.p.p. is plotted on the ordinate against time. Each point represents the mean of 100 e.p.p.s. E.p.p.s were recorded by conventional intracellular microelectrode techniques, and neuromuscular blockade was produced by bathing the preparation in high magnesium/low calcium Ringer solution. During the averaging of the e.p.p., the motor nerve was stimulated at a rate of two per s. 0.1 μ M HgCl_2 caused no detectable change in the e.p.p. amplitude; however, the addition of 1.0 μ M HgCl_2 increased the e.p.p. amplitude by 420% from the control value. The e.p.p. amplitude did not remain constant during the prolonged exposure to 1.0 μ M mercury ions; after reaching its peak amplitude, the e.p.p. amplitude eventually fell to zero. The concentration of Ca^{2+} was 0.36 mM and of Mg^{2+} , 1.1 mM. The temperature was 15 °C.



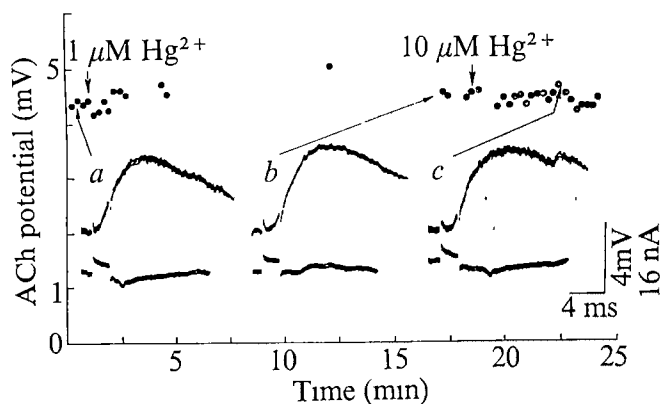


Fig. 2 Lack of a postsynaptic effect of HgCl_2 . ACh was iontophoretically applied to the endplate receptors. The amplitude of the ACh potential was plotted on the ordinate against time. The oscillogram in *a* shows the control ACh potential (upper trace) and the corresponding iontophoretic current in the lower trace. Records in *b* and *c* show that, respectively, 1.0 and 10.0 μM HgCl_2 had no obvious effect on the amplitude of the ACh potential. Each point on the plot represents the amplitude of a single ACh potential. Note the presence of a single m.e.p.p. on the falling phase of the ACh potential in *c*; 10.0 μM HgCl_2 , as in other experiments, increased the m.e.p.p. frequency. The concentration of Ca^{2+} was 0.36 mM and of Mg^{2+} , 1.1 mM. The temperature was 15 °C.

value, illustrating our consistent finding that this effect of mercury is irreversible. When this same preparation was exposed to 10.0 μM mercury, the e.p.p. at first increased further, then decreased quickly to zero.

del Castillo *et al.*⁵⁻⁷ have shown that several types of sulphhydryl reagents including oxidising agents, organic mercurials and divalent mercury ions depolarise the endplate membrane. They suggest that activation of the endplate involves a change in certain membrane sulphhydryl groups from their initial reduced form, a process essentially identical to that proposed for presynaptic transmitter release by Kosower and Werman (see ref. 3). To determine whether the effects of HgCl_2 on the e.p.p. amplitude in our experiments were presynaptic or postsynaptic in origin, the amplitude of the iontophoretic potential (ACh potential) produced by a constant amount of ACh released from a micropipette was observed before and during exposure of the preparation to HgCl_2 . Identical Ringer solutions were used during the control periods of the experiments illustrated in Figs 1 and 2.

Figure 2 contains a plot of the amplitude of the ACh potential (ordinate) against time; measurements of the amplitude were obtained before and after the application of 1.0 μM and 10.0 μM mercury ions. Figure 2*a*, *b* and *c* are representative oscillograms of these ACh potentials. The ACh pipette was highly localised at the intrinsic receptors⁸; the ACh sensitivity⁹ was quite high, the value being 787 mV nC⁻¹ for the recording made during the control period (Fig. 2*a*). Mercury caused no great increase in the amplitude of the ACh potential; the ACh potential measured in 10.0 μM mercury (Fig. 2*c*) was 4.2% higher than that measured during the control period (Fig. 2*a*). Clearly then, the massive increase in the e.p.p. amplitude observed in the experiment of Fig. 1 was due to a presynaptic effect of mercury ions.

We conclude that 1.0 μM mercury increased the amount of transmitter released from the nerve terminal after the arrival of an action potential, and that the large increase in the e.p.p. amplitude was due principally to this potentiating effect of the low concentration of mercury on evoked transmitter release. These experiments suggest that sulphhydryl groups are involved in the release of transmitter from nerve terminals. We suggest that, at very low con-

centrations of HgCl_2 , mercury dithiolate bridges form within the presynaptic nerve terminal as the presynaptic action potential arrives. In these conditions, mercury ions are presumed to act like calcium ions with regard to the evoked release of transmitter. But as the concentration of mercury ions is increased or the exposure time to a relatively low concentration of mercury ions is increased, transmitter release is blocked, presumably because the bonds between sulphur and mercury are relatively irreversible and do not allow for thiol groups to be reformed as they would in the presence of calcium ions.

The frequency of miniature endplate potentials (m.e.p.p.s) was unaffected by mercury concentrations of 1.0 μM or lower. Only at concentrations of 10 μM or more—concentrations at which the e.p.p. was usually blocked completely—did we observe an increase in m.e.p.p. frequency. Methyl mercury chloride has also been observed to produce an increase in m.e.p.p. frequency as well as neuromuscular blockade¹⁰. Thus, the action of mercury ions on the evoked release of transmitter occurs at much lower concentrations than its effects on spontaneous release. This is similar to the effects of PbCl_2 on the neuromuscular junction¹¹ except that low concentrations of PbCl_2 only decreased the e.p.p., never increased it.

It is uncertain what direct bearing the results of these experiments may have on neuromuscular disorders associated with chronic mercury poisoning. It is interesting, however, that a study¹² of methyl mercury poisoning showed that patients benefit from the administration of neostigmine, an anticholinesterase agent. Thus, although other interpretations are possible, it may be that disturbances in the release of acetylcholine from motor nerve terminals may prevail in chronic mercury poisoning.

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Effect of internal fluoride and phosphate on membrane currents during intracellular dialysis of nerve cells

It has become possible recently to isolate single neurones from molluscan ganglia by the use of proteolytic enzymes, so that the external solution can be changed easily^{1,2}. One advantage is that the absence of the axon makes the application of the voltage clamp technique to isolated neurones more effective³. We describe here a further development—an attempt to produce wide ranging changes in the ionic com-

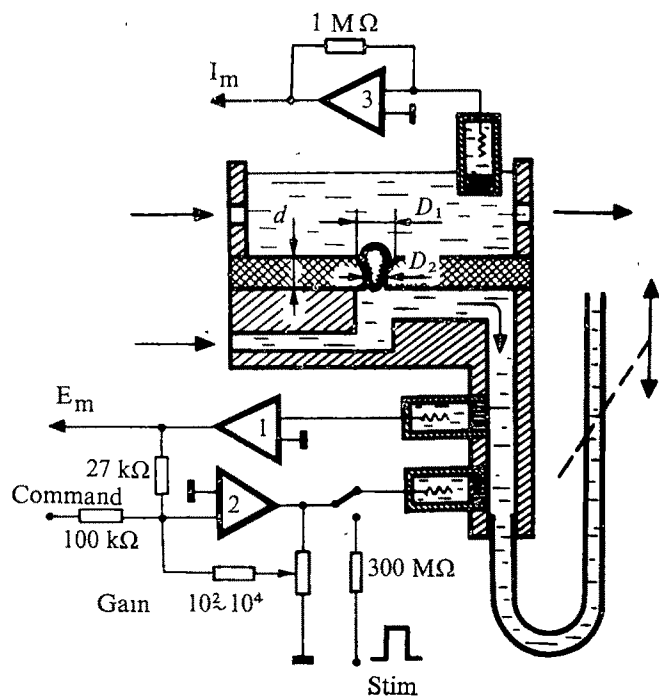


Fig. 1 Experimental design for intracellular dialysis and voltage clamping. The dimensions of the pore were: $D_1 = 200 \mu\text{m}$, $D_2 = 40 \mu\text{m}$. Polyethylene film has a thickness d of $400 \mu\text{m}$. The suction in the pore is regulated by changing the level of the outflow as shown by a vertical arrow. Electric circuits: 1, unity gain input amplifier; 2, amplifier for voltage clamping; 3, current-measuring amplifier.

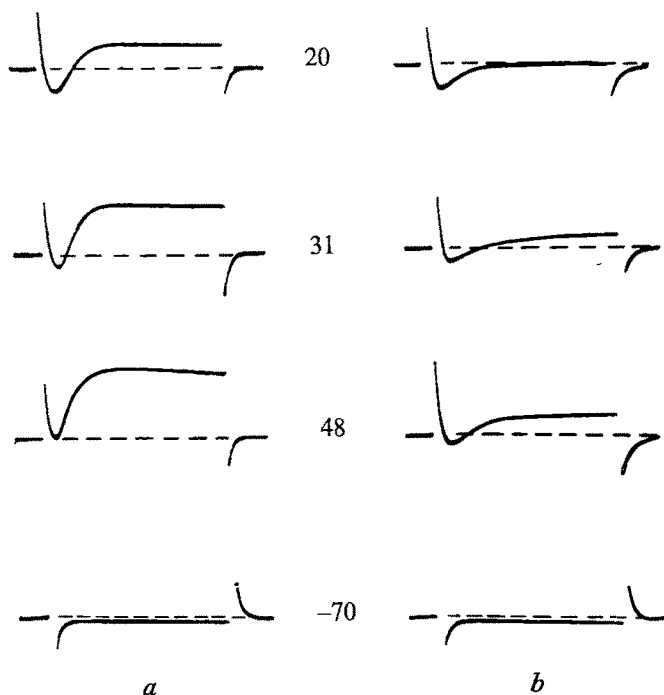


Fig. 2 Effect of a decrease in the internal potassium concentration. Potassium concentration in the lower compartment was decreased from 60 mM (a) to 2 mM (b). Osmolarity was maintained with Tris-HF (pH 7.3). Normal Ringer solution was in the upper compartment. Membrane potentials at test pulses (in mV) are indicated by numbers near the corresponding current traces. Holding potential was -40 mV . Calibrations: $4 \times 10^{-8} \text{ A}$, 10 ms.

position of the intracellular medium of neurones isolated from the snail *Helix pomatia*.

The experimental design is shown in Fig. 1. The perfusion chamber consists of two compartments separated by a polyethylene film with a conical pore. If a small negative hydrostatic pressure is created in the lower compartment, a suction effect is created in the pore. When an isolated cell is transferred to the solution in the upper chamber, it plugs the pore and becomes fixed in it. The walls of the pore are covered with an adhesive material made from Parafilm which helps to reduce leakage between the cell membrane and the walls of the pore. The part of the cell membrane that is exposed to the lower

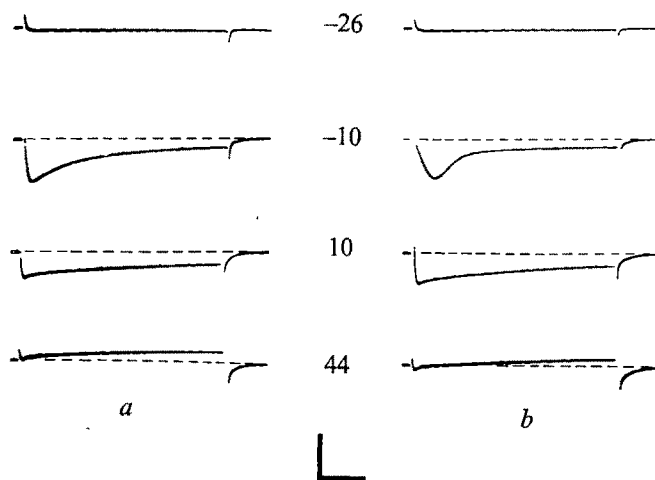
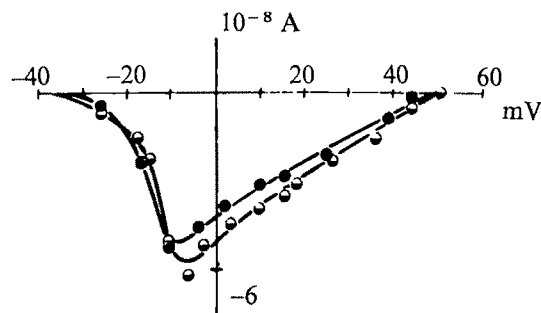


Fig. 3 Currents and $I-E$ relationship for the peaks of inward current in the cell containing phosphate anions. Solution in the lower compartment: 100 mM Tris- H_2PO_4 , pH 7.3, 20 mM sucrose. Upper compartment: normal Ringer (a, \bullet on the $I-E$ curve); Na-free solution (b, half-closed circles). Leakage was compensated for in this experiment as described in the text. Holding potential was -40 mV . Calibrations: $6 \times 10^{-8} \text{ A}$, 100 ms.

compartment is subjected to the action of calcium-free iso-osmotic (or slightly hypo-osmotic) potassium salt solution, pH 7.3, which is perfused through this compartment; this rapidly destroys the barrier properties of the membrane. (The presence of potassium is not critical in this respect since the absence of calcium itself is usually sufficient.) The upper part of the surface membrane which is in contact with normal Ringer solution (100 mM NaCl, 10 mM CaCl_2 , 2 mM KCl, 10 mM Tris-HCl, pH 7.5) in the upper compartment usually maintains its electrical excitability.

Being fixed in a pore, the cell membrane is electrically controlled with the help of the Ag/AgCl/saturated KCl electrodes connecting the solutions in both compartments to the voltage clamp device as shown in Fig. 1. Resultant membrane potential values of -20 to -30 mV can be recorded between

the two compartments showing that leakage is not more than twice as great as that normally observed in the isolated cells.

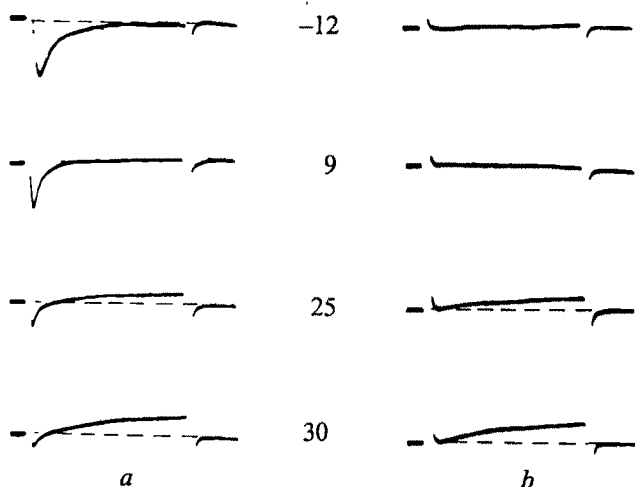
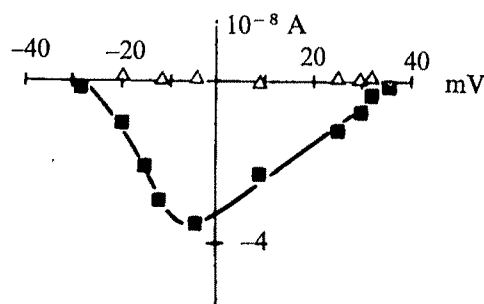
On repolarisation to a normal resting potential (-40 to -50 mV), the upper part of the membrane produces either reduced or, in many cases, full sized action potentials.

Thus we were able to dialyse the cell body and so change the intracellular ionic composition. The effectiveness of dialysis was tested first by replacing the potassium in the lower compartment by Tris. This substitution resulted in a quick (1–5 min) and marked decrease in the delayed outward current carried by potassium ions (Fig. 2).

The stability of the membrane depended largely on the kind of anions in the dialysing solution. In 100 mM KCl (or Tris-HCl) the membrane could not maintain its initial condition for sufficient time to facilitate its study. The leakage current grew continuously and the ionic currents deteriorated. NO_3^- was even less favourable in this respect. With fluoride or phosphate salts, however, the membrane showed a remarkable stability which exceeded that found during usual micro-electrode experiments on intact cells. The leakage conductance was stable for hours and did not depend on potential within the range of potential changes used. Thus we could compensate for leakage by adding to the current signal a square pulse proportional to the potential step.

The properties of the inward current were strikingly different

Fig. 4 Currents and I - E relationship for the peak inward current in the cell containing fluoride anions. Lower compartment: 100 mM Tris-HF, pH 7.3, 20 mM sucrose. Upper compartment: normal Ringer (a, \blacksquare); Na-free solution (b, \triangle). Leakage was compensated for. Holding potential was -40 mV. Calibrations: 3×10^{-8} A; 20 ms.



depending on whether fluoride or phosphate anions were introduced into the cell. When dialysed with phosphate salts the cells developed inward currents which were independent of external sodium and carried only by calcium ions. When external sodium was replaced by Tris there was no decrease in the inward current, but a slight increase could be observed, as if there was a degree of potential-dependent blocking of inward current channels by sodium (Fig. 3). In cells containing fluoride, however, the inward membrane current was carried entirely by sodium, disappearing after replacement of this ion (Fig. 4). An increase in external calcium did not abolish this effect. A cell dialysed first in the presence of phosphate salts and demonstrating calcium inward currents could be made to transport sodium by the introduction of fluoride. In neither state were the inward currents sensitive to tetrodotoxin, and the slope conductances of their steady state I - E relationships were similar, indicating that probably the same system of channels is involved in the transfer of either calcium or sodium ions. The change of the inward current channels was not completely reversible: the change back from fluoride to phosphate was followed by a sharp decrease in maximum current.

The calcium current detected in the cells containing phosphate had kinetics different from those of the sodium current in the cells containing fluoride. The latter inactivated completely, the time course being exponential (Fig. 4), whereas the former had a small, very slowly inactivating component (Fig. 3). This component may be similar to a slow calcium current recently observed in unisolated neurones⁴.

The reversal potential for this late component was 20–30 mV less positive than for the early inward current which reversed at about +50 mV (Fig. 3). This could be due to its contamination by a residual small potassium current which could be observed at high depolarisation.

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Simultaneous changes in fluorescence and optical retardation in single muscle fibres during activity

WHEN a skeletal muscle is stimulated to twitch, small changes in the optical properties of the fibres can be detected which may give information about excitation-contraction coupling. An example is provided by the experiments of Bezanilla and Horowicz^{1,2}. After treatment of muscles with Nile Blue A, fluorescence changes were found which, it was suggested, arise from changes in potential across the membranes of the sarcoplasmic reticulum (SR). Such potential changes might reflect the movement of calcium ions from inside the SR into the myoplasm, a step in the normal activation of contraction. Baylor and Oetliker^{3,4} measured changes in optical retardation, which rely on the inherent birefringence of a muscle fibre rather than the addition of an extrinsic probe. The large second component of the signal was suggested to arise from a change in SR membrane potential. These explanations are plausible since small changes in both extrinsic fluorescence

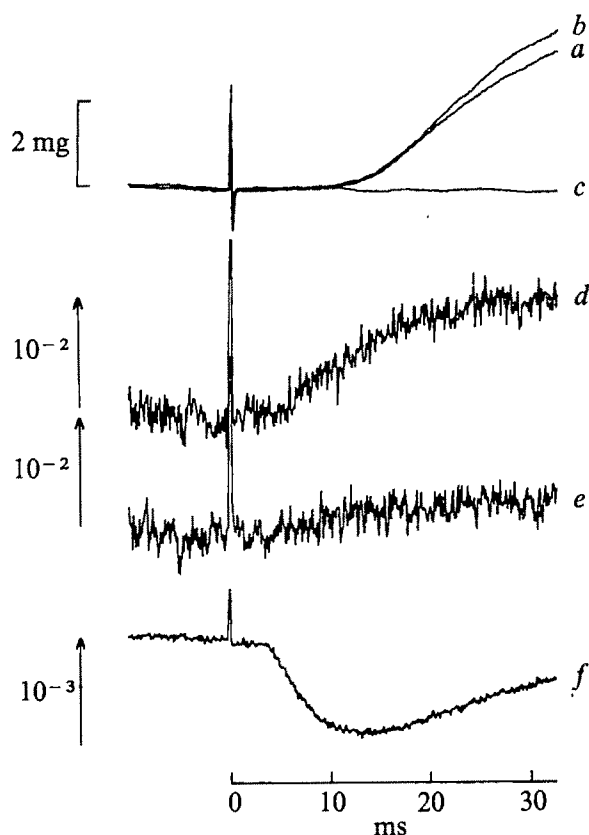


Fig. 1 Tension, Nile Blue A fluorescence and optical retardation in a single muscle fibre during a twitch. Records *a-c* show twitch tension recorded in Ringer (*a*), 18 min after introducing Ringer + Nile Blue A ($0.0002 \text{ mg ml}^{-1}$) (*b*), and 10 min after replacing with 270 mM NaCl Ringer + dye ($0.0002 \text{ mg ml}^{-1}$) to block movement (*c*). The tension recorded in isotonic solution was relatively low since the fibre was small, diameter $56 \mu\text{m}$, and was stretched to 1.55 times slack length. Records *d-f* show optical measurements made after the hypertonic Ringer was introduced. *d*, Change in fluorescence expressed as change in light intensity divided by resting fluorescent light intensity, using light of $570 \pm 30 \text{ nm}$ to illuminate the fibre (determined by primary filter) and measuring light of wavelengths longer than 630 nm (determined by secondary filter). *e*, Fluorescence control expressed as change in light intensity divided by the resting intensity used in *d*, obtained by placing the secondary filter between the primary filter and the fibre. *f*, Change in optical retardation using light of $570 \pm 30 \text{ nm}$. For records *a*, *b* and *f*, 16 traces were signal averaged; for *c*, *d* and *e*, 32 traces were averaged. In each record the fibre was stimulated 10 ms after the beginning of the sweep as indicated by the stimulus artefacts. An upward deflection in the optical traces represents an increase in light intensity, as indicated by the arrows. Temperature, $12-13^\circ\text{C}$.

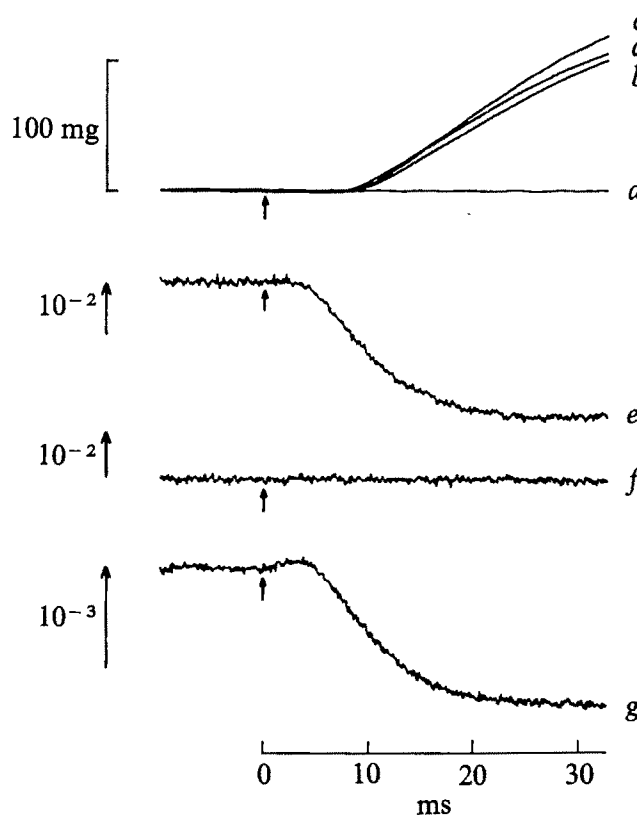
and optical retardation are known to accompany voltage changes across neuronal membranes⁵⁻⁸. An important test of these hypotheses is to compare the fluorescence and retardation signals from the same fibre, to see if they follow the same time course.

Single fibres from the semitendinosus or ilio-fibularis muscle of English frogs (*Rana temporaria*) were isolated and mounted horizontally in a narrow chamber with glass sides. One tendon end was connected to an RCA 5734 transducer for monitoring tension. The chamber was then placed on an optical bench (Spindler-Hoyer) where the light from a tungsten-halogen source could be focused on the fibre by a long working distance objective ($\times 32$, N.A. 0.60). An identical objective was placed on the opposite side to collect the transmitted and emitted light. Light collected from 0.3 mm of fibre was measured by a photodiode (PV-100, E.G. & G., Inc.). The tension transducer and the photodiode were connected to Tektronix 3A9 amplifiers, bandwidth d.c. to 3 kHz, and the outputs were sampled and

signal averaged on-line by a PDP-8/e computer. Measurements of fluorescence were made using a wide-band interference filter (primary filter) placed between the light source and the muscle fibre and a longer wavelength cut-on filter (secondary filter, Schott R.G. series) placed between the fibre and the photodiode. Optical retardation was measured with two polarisers placed on either side of the fibre, one at -45° and the other at $+45^\circ$ with respect to the fibre axis⁴. A shutter limited the exposure of the fibre to light. For data collection the fibre was stimulated externally with 0.2 ms pulses at a rate of $0.1-0.2 \text{ s}^{-1}$. Only fibres that gave all-or-nothing mechanical and optical responses were used. Optical recording was usually done about 1 mm from the cathod. The Ringer solution contained 120 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 , 2.15 mM Na_2HPO_4 and 0.85 mM NaH_2PO_4 , pH 7.1 (ref. 9). Mechanical movement was sometimes blocked by increasing the NaCl concentration to 270 mM, thus making the tonicity of the solution about 2.2 times normal.

In the first experiments with Nile Blue A it became apparent that single fibres do not survive well in the concentration used successfully on whole muscle^{1,2}, 0.02 mg ml^{-1} . In one experiment a 20-fold dilution produced a potentiation of the twitch of about 50% in 10 min, which was

Fig. 2 Tension, indodicarbocyanine fluorescence and optical retardation during a twitch. Records *a-d* show twitch tension in Ringer (*a*), 14 min after introducing Ringer + 0.002 mg ml^{-1} of the dye (*b*), 13 min after returning to normal Ringer (*c*), and 5 min after replacing with 270 mM NaCl Ringer (*d*). The fibre was in the dye solution for 19 min, then in isotonic Ringer for 14 min before hypertonic Ringer was introduced. Records *e-g* show singlesweeps of changes in fluorescence (*e*), fluorescence control (*f*), and changes in optical retardation (*g*), all made after hypertonic Ringer was added. For fluorescence measurements the primary filter passed light of $570 \pm 30 \text{ nm}$ and the secondary filter passed light of wavelengths longer than 665 nm . Optical retardation was measured using light of $570 \pm 30 \text{ nm}$. Stimulation time indicated by arrows. Fibre diameter $151 \mu\text{m}$; stretched 1.32 times slack length. Temperature $14-16^\circ\text{C}$. The later portion of the optical retardation signal in this experiment is different from that shown in Fig. 1, the reason probably being related to degree of stretch (unpublished results of S.M.B. and H.O.).



reversed within 15 min after removing the dye. Since a 100-fold dilution, $0.0002 \text{ mg ml}^{-1}$, had little or no potentiating effect, this concentration was used for optical recording. Record *a* in Fig. 1 shows tension recorded in Ringer solution, record *b* in Ringer plus dye, and record *c* after 270 mM NaCl Ringer plus dye was introduced to minimise movement artefacts on the optical traces. Signal-averaged records of fluorescence (*d*) and of optical retardation (*f*) were each taken with the fibre in hypertonic solution. The initial phases of both signals are qualitatively similar to the signals observed at normal tonicity, except that hypertonicity reduced the maximum rate of change by a factor of 4 for optical retardation⁴ and about 3 for fluorescence. The change in light intensity in record *d* represents primarily a change in fluorescent light from Nile Blue A since the signal was not seen without the dye and was greatly reduced when the secondary filter was placed between the primary filter and the muscle fibre, record *e* ('control' trace). Similarly, the signal in record *f*, corresponding to Baylor and Oetliker's second component⁴, represents a change in optical retardation since it can be reversed by adding an appropriate amount of compensation (not shown). The conclusion from the experiment is that the fluorescence and optical retardation signals are similar in appearance, at least initially, but that the noise level of the fluorescence traces *d* and *e* is too high to permit accurate quantitative comparison. For this reason, it was desirable to find a fluorescent dye that would give a larger signal, without otherwise affecting the fibre.

A marked improvement in the signal-to-noise ratio was obtained with an indodicarbocyanine dye (dye 122 in ref. 7) which could be used in concentrations up to 0.002 mg ml^{-1} with little effect on the twitch. Record *a* in Fig. 2 shows tension recorded in Ringer; *b*, after introducing the dye; *c*, after returning to Ringer; *d*, after replacing with 270 mM NaCl Ringer. Record *e* shows a single fluorescence trace (that is, not signal averaged), *f* shows a single fluorescence control trace and *g* shows a single optical retardation trace, all taken with the fibre in hypertonic solution. Apart from the slight hump in trace *g*, which occurs just before the downward deflection, traces *e* and *g* are remarkably similar. The cause of the hump, which was seen only when dye was used, is not understood. In this experiment the change from isotonic to hypertonic solution reduced the maximum rate of change of both optical signals by a factor of 6.

Since a merocyanine dye (dye I of ref. 7, available as Merocyanine 540, Eastman Organic Chemicals) has been reported to give a fluorescence signal originating mainly from T-system membrane¹⁰, it was interesting to try this dye as well. Figure 3 shows results from two experiments. *A* shows signal-averaged records obtained in isotonic Ringer plus dye. Trace *a* shows tension, *b* shows fluorescence, and *c* gives the control for fluorescence. Although movement artefacts became apparent once tension started to develop, it is clear that the fluorescence signal was early and transient, unlike the fluorescence signals obtained using the other dyes. In other experiments, the merocyanine signal propagated with a velocity comparable with that expected for an action potential, making it unlikely that it represents some sort of movement artefact. This merocyanine dye is toxic⁷ to single muscle fibres, however. Similar experiments in hypertonic solution were even more difficult to carry out. Figure 3*B* shows our best attempt. Record *a* indicates tension, *b* and *c* show fluorescence and fluorescence control, respectively, and *d* shows optical retardation. It is apparent that the change in fluorescence precedes the large change in optical retardation, a finding consistent with the idea that merocyanine is an indicator of surface and T-system membrane potential.

The general conclusion from these experiments is that the second component of the optical retardation signal and the fluorescence signal from Nile Blue A or an indodicarbo-

cyanine dye have approximately the same time course, making it likely that they are indicators of the same basic process or processes. Since changes in membrane potential can give rise to both types of optical signals, the similarity of the signals might be considered as evidence for such a mechanism in muscle. If so, the arguments of Bezanilla and Horowicz² and Baylor and Oetliker⁴ make the SR membrane the likely candidate. Although this view is attractive,

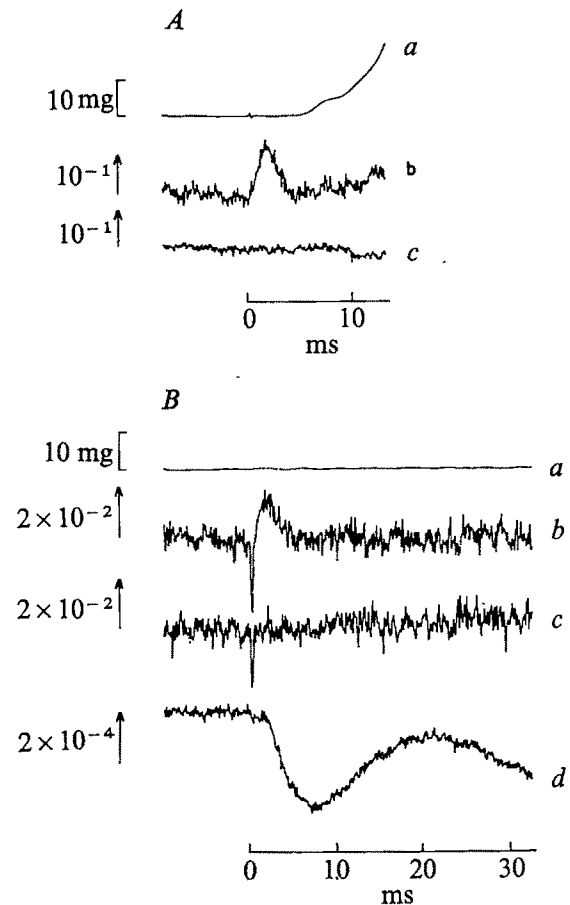


Fig. 3 Tension, fluorescence and optical retardation using a merocyanine dye. *A*, records made in isotonic Ringer saturated with the dye. Trace *a* shows tension, *b* shows change in fluorescence and *c* shows the fluorescence control; four sweeps were signal averaged for *a* and *b*, 16 for *c*. The traces were cut off at the time when large movement artefacts started to develop on the optical traces. Fibre diameter and stretch were not recorded. *B*, records obtained from a different fibre in 270 mM NaCl hypertonic Ringer without dye following 30 min in normal Ringer + dye. The dye in the soaking solution was dissolved in ethanol and Pluronic F-127 (BASF Wyandotte, as described in ref. 7) before being added to Ringer to give a final concentration of 0.018 mg per ml of dye, 0.2% ethanol and 0.02% Pluronic. Just before use the solution was filtered through a 0.22- μm Millipore filter. Trace *a* shows tension, *b* shows fluorescence change, *c* gives the fluorescence control and *d* gives the change in optical retardation using wavelengths longer than 665 nm; eight sweeps were signal averaged for *a*, *b*, *c* and four sweeps were averaged for *d*. Fibre diameter 123 μm ; stretched 1.31 times slack length. For the fluorescence measurements in *A* and *B* the primary filter passed light of $570 \pm 30 \text{ nm}$ and the secondary filter passed wavelengths longer than 630 nm. All solutions were bubbled with N_2 . These experiments were carried out at room temperature, 19°C in *A* and 21°C in *B*.

other explanations should also be considered. For example, optical signals might accompany an increase in myoplasmic calcium or a possible change in SR volume related to calcium release. In any event it is encouraging that fluorescence as well as birefringence methods can be made to work on single muscle fibres without the use of signal

averaging, and that different dyes might be used to follow different steps in muscle activation.

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Membrane noise in slowly adapting stretch receptor neurone of lobster

ANALYSIS of membrane noise in excitable membranes may yield information about the kinetics governing the conductance control of various ionic channels. Such analysis has been applied to noise arising from conductance fluctuations in, for example, the potassium system^{1,2} or the system of postsynaptic ionophores^{3,4}. This report deals with noise that seems to be generated in the sodium channel of the slowly adapting stretch receptor neurone of lobster. We recorded this noise as current noise under voltage clamp, and subsequently analysed it with respect to its covariance and spectral density function. A particularly low degree of Na inactivation at polarisations down to firing threshold was inferred from these analyses.

We used isolated cells mounted in a chamber continuously perfused with solutions of desired composition at 18 °C. For voltage clamp, the same low resistance (5–8 MΩ) micro-electrode, filled with 3 M KCl, was used for measuring the membrane potential and passing the potential controlling currents. This we did by subtracting any potential artefact due to current flow in the electrode from the output signal of the input amplifier before passing it on to the control amplifier. Tests with electronic circuits simulating the membrane and microelectrode showed that a satisfactory signal subtraction of this kind could be achieved for control currents up to 5 nA in a frequency range of 0–250 Hz. After bandpass filtration (cut-off frequencies at 1.6 and 350 Hz), the recorded current noise was processed on a PDP-15 computer for determination of the covariance function and its Fourier transform (spectral density function) between 2 and 400 Hz.

A distinct voltage noise was seen in the slowly adapting stretch receptor neurone of lobster at subthreshold conditions. This noise increased with increasing depolarisation and became indiscernible only after polarisation beyond resting potential. Under voltage clamp the corresponding current noise exhibited a similar polarisation dependence (Fig. 1a and b). The membrane noise was abolished by addition of 120 nM tetrodotoxin or by complete substitution of choline or Tris for extracellular Na⁺ (Fig. 1c and d). The noise was unaffected, however, by 20 mM tetraethyl-

ammonium (Fig. 1e and f), whose ability to block the K channel was manifested by elimination of the after-hyperpolarisation of the action potential. These pharmacological tests show that the Na system was involved in the generation of noise in our preparation.

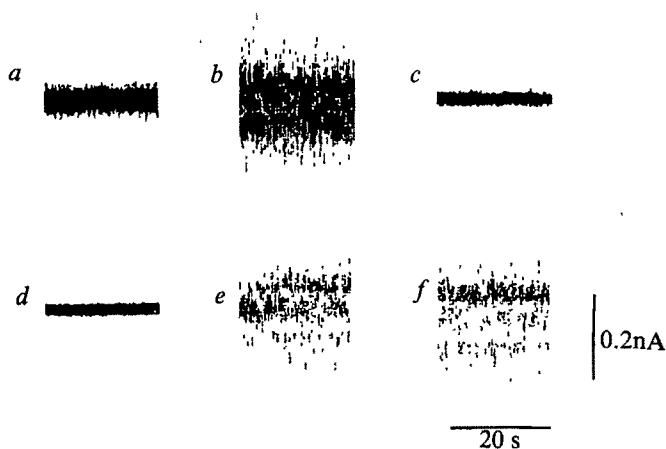
For a more detailed analysis of the noise generating process, covariance and spectral density functions were computed for current noise recorded at different levels of membrane polarisation. The results of such analyses showed that for more moderate depolarisations the computed covariance and spectral density functions agreed well with theoretical values typical of a first-order noise generating process (Figs 2 and 3). For depolarisation close to firing threshold, however, the computed covariance function deviated in a sinusoidal fashion from an exponential development (Fig. 2), and the spectral density function formed a hump superposed on the simple $[1 + (f^2/f_0^2)]^{-1}$ relationship (Fig. 3). It also seemed that with depolarisation there was an increase in time constant of the covariance function and a decrease in half-power frequency (Fig. 3) of the spectral density function. Finally, the noise variance (that is the covariance function at zero time) increased at an increasing rate with depolarisation.

The more complicated behaviour of the computed covariance and spectral density functions in near-threshold conditions was interpreted to indicate the presence of some periodic signals in the noise. In our preparation such signals could arise in connection with a subthreshold repetitive activity in peripheral cell regions where voltage was not well controlled and will not be considered here.

With regard to the first-order process which seems to underlie noise generation at all lower, but presumably also at higher depolarisations, it is assumed to be identical with the gating process of the Na channel. This gating process can be envisaged as some kind of state transition in channel gating elements, whereby the transition intensity obeys first-order kinetics^{5,6}. Measurements of so-called gating currents have verified the presence of first-order kinetics, at least as far as state transitions in the Na activating system are concerned^{7,8}.

For a more detailed interpretation of our noise recordings we presume that the Na channel is gated by an activating (*m*) and by an inactivating (*h*) system⁹. Provided that both of these systems are subject to random state variations, which is likely from thermodynamic principles, both will generate noise components (henceforth called *m* and *h*-noise

Fig. 1 Membrane current noise recorded at resting potential (–60 mV) (a); at 8 mV depolarisation from resting potential (b); at 8 mV depolarisation in the presence of 120 nM tetrodotoxin (c); at 14 mV depolarisation after substitution of choline for extracellular Na⁺ (d); at 8 mV depolarisation in the absence (e), and in the presence (f) of 20 mM tetraethylammonium. The current calibration represents an approximation.



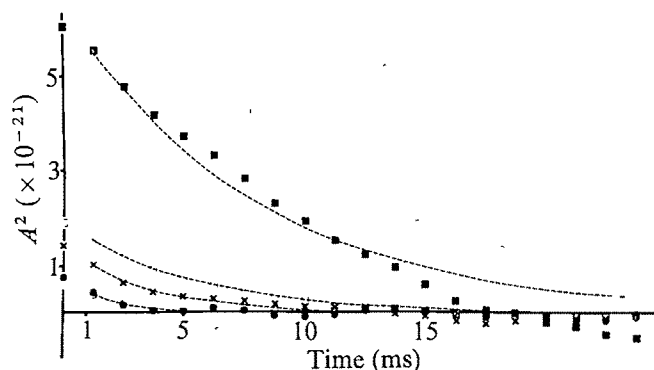


Fig. 2 Covariance function (ordinate) computed for current noise recorded at resting potential (-70 mV) (\bullet) and at 12 mV (\times), 13 mV (\square), and at 14 mV (\blacksquare) depolarisation from resting potential, and at 16 mV depolarisation in the presence of 120 nM tetrodotoxin (\circ). ---, Theoretical values which are proportional to $\exp(-|\Delta t|/\tau)$, where Δt represents the time lag (abscissa) and τ a time constant whose value varies between 1.6 and 7.9 ms.

components) which then combine in the formation of the membrane noise.

If it is assumed that, in subthreshold conditions, the time constants for state transitions in the m and h system are of the order of 0.1 – 0.3 ms, and 1 – 10 ms, respectively⁹, it follows that the bandwidth of the m noise will extend well beyond 500 – $1,500$ Hz, whereas that of the h noise will stay below 150 Hz. This would mean that in the present recording conditions most of the high frequency m -noise components had no chance of appearing in the noise recordings. In consequence, the computed covariance and spectral

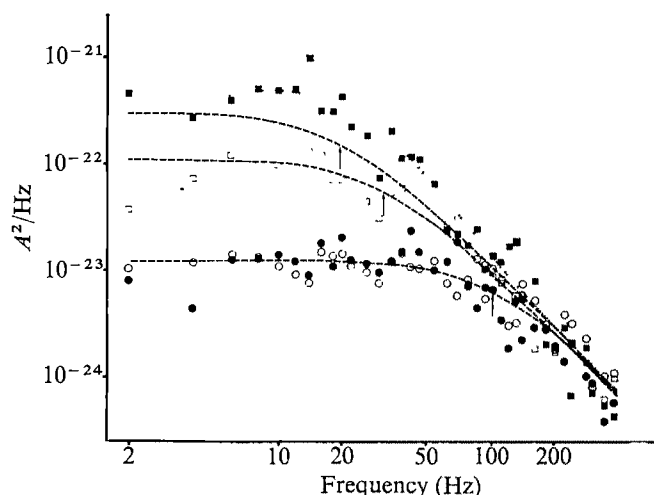


Fig. 3 Spectral density function (ordinate) computed for current noise recorded at resting potential (-70 mV) (\bullet) and at 13 mV (\square), and 14 mV (\blacksquare) depolarisation from resting potential, and at 16 mV depolarisation in the presence of 120 nM tetrodotoxin (\circ). ---, Theoretical values which are proportional to $[1 + (f^2/f_0^2)]^{-1}$, where f represents the frequency (abscissa) and f_0 the half-power frequency whose value varies between 20 and 100 Hz (arrows). The dotted line is fitted by eye.

density function can contain no information regarding the kinetics of the m system. The presence of low frequency m -noise components in the recorded noise may, however, explain the steep increase of the noise variance with increasing depolarisation. With respect to the h -noise, because of its narrow bandwidth it would be expected to pass the filtering circuits of the recording system fairly undistorted and therefore be able to reveal the kinetics of the h system. For this reason it is held that the voltage dependent changes in time constant of the covariance function,

or half-power frequency of the spectral density function are true indications of the voltage dependence of the kinetics of the h system. It is therefore concluded that in our preparation the time constant of the h system is increasing monotonously as the membrane potential is lowered towards firing threshold. This indicates a low degree of Na inactivation in near-threshold conditions. The reason for this could be an especially fast transition from 'closed' to 'open' conformation of the h system, as is occasionally found in particularly non-accommodating nerve fibres^{10,11}.

Functionally, such setting of the Na channel gating system would provide for the occurrence of a subthreshold steady-state Na conductance¹², which might explain the non-adapting mode of firing in our preparation¹³. This hypothesis is corroborated by the fact that a distinct Na noise is not demonstrable in the fast adapting stretch receptor neurone of lobster.

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Subthreshold membrane currents in slowly adapting stretch receptor neurone of lobster

In the slowly adapting stretch receptor neurone of lobster, application of tetrodotoxin (TTX) causes a simultaneous hyperpolarisation and decrease in input resistance¹. These effects have been attributed to a possible increase in potassium conductance. They could, however, be explained by elimination of a subthreshold steady-state sodium conductance which, in this case, might underlie the cell's repetitive mode of firing². To examine this possibility, we made a voltage clamp study of the subthreshold steady-state membrane currents of the slowly adapting stretch receptor neurone of lobster. The results indicate the existence of a subthreshold steady-state Na current which to a large extent seems to be balanced by a leak type current.

We used isolated cells mounted in a chamber continuously perfused with solutions of desired composition at 18°C . Membrane potential and membrane currents were recorded under voltage clamp using a single, low resistance (5 – 8 M Ω) intrasomal microelectrode filled with 3 M KCl (ref. 3). This method permitted measurement of steady-state membrane currents that did not exceed 5 nA. Such currents were encountered in a voltage range of 10 – 15 mV in either direction from resting potential (Fig. 1). Steady-state values of the membrane current were determined 10 – 15 s after each polarisation adjustment.

Typical current measurements in the absence and presence of TTX (Fig. 1) show that in control conditions the curve relating membrane current to membrane polarisation had a negative slope starting at depolarisations of 5 – 7 mV. After treatment with 120 nM TTX the current-voltage curve lost this negative slope and became steeper. At the same time, its voltage intercept was shifted in a

negative direction by about 5 mV. In agreement with earlier findings these changes imply a hyperpolarisation as well as a decrease in input resistance in the absence of any injected current.

To see whether these changes originated only in the elimination of a Na conductance, all extracellular Na^+ was replaced by either choline or Tris. In both cases a current-voltage curve was obtained very similar to that encountered after TTX treatment, although potentials were more negative by about 5 mV. This curve underwent no further changes if TTX was added to the Na-free solutions. Thus TTX did not affect the K channel or the leak channel in our preparation and so its effect on the current-voltage relationship, as described above, must have been due to elimination of a specific Na conductance.

For an estimate of the magnitude of this conductance, the current-voltage curves obtained in the absence and presence of TTX, respectively, were subtracted from each other. The resulting relationship between Na current and membrane polarisation (Fig. 1) shows that Na conductance was appreciable in resting conditions and that it did not vanish until the cell was hyperpolarised by at least 10 mV; in depolarising conditions (down to firing threshold) the Na conductance increased at an increasing rate.

To study the nature of the membrane current, I_x , which balances the Na current in resting conditions, cells were exposed to 20 mM tetraethylammonium (TEA) after pre-treatment with 120 nM TTX. In the absence of any injected current this led to a slight depolarisation (2–3 mV) and a minor increase (10–20%) in the input resistance. These changes show—presuming that TEA eliminated the K conductance completely—that in resting conditions only 20–30% of I_x can be attributed to a specific K current. The remaining and greater part of I_x therefore seems to be a leak type current. The ions carrying this current presumably include not only K^+ and Cl^- but also Na^+ . The presence of a Na component in the leak current is suggested by the 5 mV hyperpolarisation that arises in response to removal of all extracellular Na^+ after preceding blockage of the specific Na channels by means of TTX.

Studies of membrane noise³ have suggested that the slowly adapting stretch receptor neurone of lobster has a small tendency for Na inactivation in subthreshold conditions. This type of permeability regulation probably explains the presence of a subthreshold steady-state Na conductance. The functional significance of such a conductance may be twofold. First, as it increases with depolarisation it increases the input resistance of the cell and thus reduces the load on the generator current source. Second, owing to its existence,

Fig. 1 Relationship between membrane current (ordinate) and membrane polarisation (relative to resting potential at -60 mV, abscissa) in the absence (▼), and presence (●) of 120 nM TTX. The relationship between Na current and membrane polarisation (—) was obtained by subtracting the curves I_x and I_m from each other.

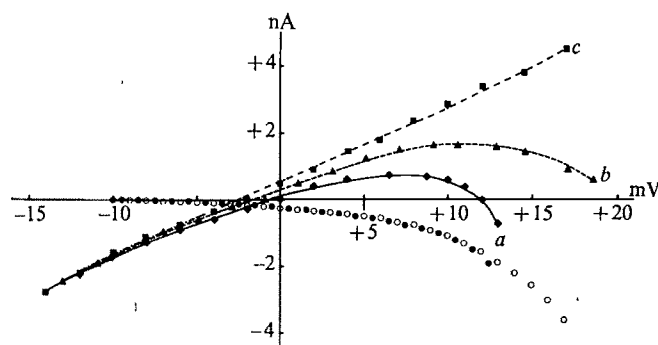
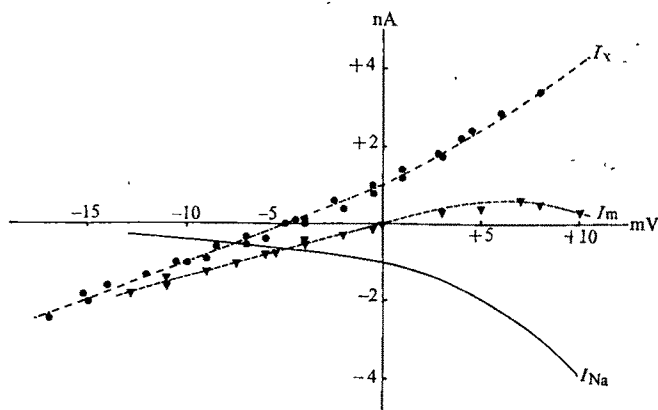


Fig. 2 Relationship between membrane current (ordinate) and membrane polarisation (relative to resting potential at -62 mV, abscissa) in control conditions (◆) and after application of TTX to the axon beyond 300–400 μm (▲), and beyond 100 μm from the cell body (■). Difference between curves a and b; ●, difference between curves b and c.

conditions may be fulfilled for the repetitive mode of firing² that is typical of the preparation we used. There is evidence for this latter assumption in the fact that no subthreshold steady-state Na conductance exists in the fast adapting stretch receptor neurone of lobster.

In an attempt to localise the site of origin of the intrasomally detectable subthreshold steady-state Na current, TTX was applied to the axon at various distances from the cell body. As a rule, this resulted in a decrease of the inward component of the membrane current (Fig. 2), indicating that a subthreshold steady-state Na current is generated in the initial parts of the axon as well as in the soma. There was a considerable decrease in inward current even when TTX was applied to relatively distant axonal segments, as shown in Fig. 2. Application of TTX to the axon 300–400 μm from the cell body and beyond gave a marked decrease in inward current. This decrease equalled the further reduction of inward current obtained when extending the treatment about 200 μm towards the cell body. Considering the cable properties of the axon this means that the axonal segments beyond a distance of 300–400 μm from the cell body could generate a stronger inward current than more proximal axonal segments. This may be related to the presence of a spike-trigger zone with particularly developed pacemaker properties about 300–500 μm out on the axon^{4,5}.

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Plasma membrane-associated increase in guanylate cyclase activity in regenerating rat liver

CYCLIC GMP has been postulated as one of the intracellular mediators triggering cell growth¹. Exogenously added cyclic GMP or its derivatives are liable to induce a substantial increase in DNA synthesis^{2–4} and to promote growth^{2,5}. Cultured, non-transformed fibroblasts⁶ and lymphocytes¹ exist in one of two reversible growth states, a state of rapid

proliferation and a state of relative quiescence and increased levels of cyclic GMP are correlated with the transition from the resting to the growth state^{1,3}. In these systems, a variety of mitogenic agents have been shown to initiate proliferation of quiescent cells and this effect seems to be accomplished by interaction with the cell surface¹. Therefore, if an increase in cyclic GMP levels were to act as the intracellular signal triggering cell proliferation, its formation and/or degradation should be linked to events occurring in the plasma membrane. Indeed, a membrane-bound guanylate cyclase (GTP pyrophosphatase, cyclising, EC 4.6.1.2.) of cultured fibroblasts has been found to be activated by a fibroblast growth factor⁷.

Partial hepatectomy induces the remaining liver cells to undergo a change from a resting to a proliferating state similar to the one cited above for cultured cells⁸, and the regenerating liver after partial hepatectomy presents a well defined model system for studying cell proliferation *in vivo*. In the normal hepatocyte, however, most of the guanylate cyclase activity is recovered in the cytosol⁹. We therefore examined the possibility that an altered sub-cellular distribution of guanylate cyclase might be found in proliferating liver cells. Data presented show an increase in guanylate cyclase activity in liver homogenates after partial hepatectomy, mainly due to an increase in plasma membrane-associated activity.

We measured cyclic GMP formation in the total homogenate (for details of preparation, see Fig. 1) of regenerating rat liver at different times after partial hepatectomy. An increase in guanylate cyclase activity was observed within 16 h, reaching a maximum 24 h after the operation (Fig. 1). This increase in guanylate cyclase activity correlates with the increase in proliferating activity⁸. At 4 d after hepatectomy, when the regeneration is nearing completion, the elevated cyclic GMP formation returned to control values.

In the quiescent liver, guanylate cyclase is mainly detectable in the soluble fraction (Table 1). At 3 d after partial hepatectomy, the activity of the soluble guanylate cyclase did not change, whereas the plasma membrane-associated enzyme activity rose to about 11 times the value of sham-operated rats. Essentially the same changes were observed when the activities were expressed as pmol cyclic GMP formed per min per g liver (Table 1). Triton X-100 stimulates guanylate cyclase activity in a number of systems^{7,9,11}. Treatment of plasma membrane fractions from normal and regenerating liver with this detergent (Triton:protein=2:1, w/w; 10 min at 0 °C) increased cyclic GMP formation 2.5–2.9 and 2.4–2.6-fold, respectively (three experiments). Thus, approximately the same increase in plasma membrane-associated guanylate cyclase activity was observed in the presence and absence of detergent. The technique used for the isolation of liver plasma membranes, aimed at obtaining the highest possible purity, makes losses of material inevitable. Using the recovery of 5'-nucleotidase (a marker enzyme for liver plasma membranes¹³) as reference, the yield of plasma membrane material in our preparations is about 11%. Table 1 indicates that more than 85% of the increased guanylate cyclase activity in regenerating liver should be localised in the surface membranes.

Rapidly growing tissue is found also in hepatoma tumours. In previous reports, no correlation between cyclic GMP levels and growth rate of hepatoma tumours was found, although cyclic GMP concentrations tended to be higher in hepatoma cells compared with normal liver^{14,15}. We examined guanylate cyclase activity in four hepatoma lines: Morris hepatomas 7777, 9618A₂, 7800 and 9121, the first two lines being rapidly-growing, the others medium-growing tumours. No detectable activity (less than 0.8 pmol cyclic GMP formed per min per mg protein) was found in three lines and an increased activity with respect to normal

liver in line 9121 (25.6±2.8 pmol per min per mg protein; three experiments). Treatment with Triton X-100 did not elicit detectable activity in 7800, 9618A₂ and 7777 lines, although cyclic GMP formation was moderately stimulated in 9121 line (1.6-fold). Addition of hepatoma homogenate without detectable guanylate cyclase activity to normal liver or to the 9121 line did not reveal the presence of enzyme inhibitors. We postulate that a normally functioning guanylate cyclase is essential for growth regulation.

In young adult rats as used in the present experiments,

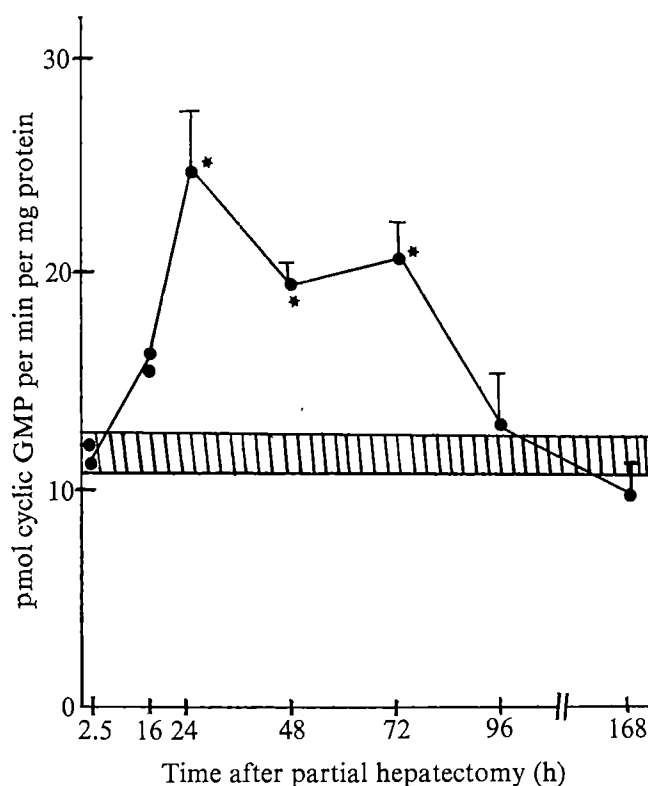


Fig. 1 Cyclic GMP formation in liver homogenates at various times after partial hepatectomy. Female Buffalo rats (150–200 g) were fed Altromin R, containing 19% protein, and water *ad libitum*. For partial hepatectomy two-thirds of the liver were removed. Sham-operated animals were used as controls. All surgical operations were carried out under ether anaesthesia in the morning between 0800 and 0900. For determinations of guanylate cyclase activity in the total homogenate, in the soluble and crude particulate fraction 0.5 g liver was homogenised in 3 volumes 10 mM Tris-HCl buffer, pH 7.4. Soluble and crude particulate fractions were obtained by centrifugation of the homogenate at 105,000g for 60 min. The pellet was washed once in the same buffer. Plasma membranes were prepared by a modified procedure of Neville's method¹⁰. The total homogenate and the soluble fraction could be stored for several weeks at –40 °C without loss of guanylate cyclase activity. The guanylate cyclase bound to the membrane, however, was highly unstable; the activity of a plasma membrane fraction stored for 2 d at –40 °C decreased by 50%. Rats were killed at the times indicated after partial hepatectomy and suitable dilutions (30–95 µg protein) of a 1:3 (w/v) liver homogenate assayed for guanylate cyclase activity as described previously¹¹ with two modifications: 0.1 mM papaverine was included in the reaction mixture to inhibit cyclic nucleotide phosphodiesterase activity; as rather low activities had to be measured in some fractions, the eluate of the Al₂O₃ columns was further chromatographed on Dowex 1×8 (formate form)¹². This step was very effective in lowering the blank values, which did not exceed 0.006% of the total radioactivity. Preliminary experiments showed that the MnCl₂ concentration used (8 mM) gave maximum activities in control and regenerating liver. Mean ± s.e.m. is given for 4 to 11 animals except for the 2.5 and 16-h values, where the figures for 2 animals are shown. Mean ± s.e.m. for 8 sham-operated rats is indicated by the horizontal bar. There were no differences between sham-operated animals killed 1–4 d after surgery. *Values significantly different from controls ($P < 0.005$).

Table 1 Cyclic GMP formation in various fractions derived from sham-operated and regenerating liver

	Guanylate cyclase activity (pmol per min per mg protein)			Guanylate cyclase activity (pmol per min per g liver)		
	Sham operation	Partial hepatectomy	% increase	Sham operation	Partial hepatectomy	Increase*
Total homogenate	11.7±0.7	20.8±1.7	77	1,567±81	2,892±221‡	1,325
105,000g supernatant	28.9±4.0	30.0±2.4	—	1,879±198	2,190±168	—
105,000g pellet	3.6±0.7	19.8±4.5†	450	182±27	1,439±246†	1,252
Plasma membrane fraction	12.1±0.8	141±26‡	1,065	9.4±0.6	135±21‡	126

* Increase in guanylate cyclase activity expressed as pmol cyclic GMP per min per g liver.

Rats were killed 72 h after partial hepatectomy. This time was chosen to minimise possible nonspecific effects of surgery. The plasma membrane fraction was comprised of fractions M₁ and M₂ from modified Neville's¹⁰ method. Aliquots of a liver homogenate and of the various fractions which were in 10 mM Tris-HCl buffer (pH 7.5) were assayed for guanylate cyclase activity. Three dilutions (30–95 µg protein for total homogenate and supernatant, 40–140 and 16–100 µg for the 105,000g pellet and the plasma membrane fraction, respectively) of all fractions were tested to ensure that the reaction velocity was proportional to enzyme concentration. No measurable hydrolysis of added cyclic GMP by phosphodiesterase occurred in any assay. Values are means ± s.e.m. for 4–11 animals. Difference significant † *P* < 0.001 (‡ *P* < 0.005) from sham-operated controls.

DNA synthesis starts to increase 12–15 h after partial hepatectomy, peak values being attained at 19–25 h (refs 8 and 16). Mitosis follows DNA synthesis by about 8–9 h (ref. 8) and it has been estimated that about 40% of the parenchymal cells divide between 30 and 40 m (ref. 16). Thus the timing of the observed increase in guanylate cyclase activity correlates well with the onset of proliferative activity after partial hepatectomy, indicating that the induction of liver cell proliferation is intimately connected with increased activity of this enzyme. Fast transient increases in cyclic GMP concentrations have been reported to occur in several *in vitro* systems^{1,3,7} after administration of mitogenic agents, which presumably interact solely with the cell surface. The fact that the increment is predominantly, if not exclusively, located in the plasma membrane also indicates that in the regenerating liver cyclic GMP acts as signal to mediate growth-initiating signals¹⁷ from the cell surface to intracellular structures (for example, the nucleus). In agreement with this hypothesis, a guanylate cyclase associated with a plasma membrane fraction of mouse fibroblasts was found to be activated by a fibroblast growth factor⁷.

During the preparation of this manuscript, Kimura and Murad¹⁸ reported on an increase in particulate guanylate cyclase activity in regenerating liver. These authors describe a less than twofold increase in guanylate cyclase activity in a plasma membrane fraction of regenerating rat liver compared with the more than 11-fold increment observed here; and we cannot confirm a decrease in the soluble enzyme activity during liver regeneration. Kimura and Murad measured an increased enzyme activity in Morris hepatoma 3924A. Using Morris hepatoma 9121 we also measured increased guanylate cyclase activity, but in three other lines (7777, 7800, and 9618A₂) no enzyme activity could be detected. These contradictory findings in Morris hepatomas demonstrate that no simple correlation between proliferation of normal tissue and malignant growth is possible.

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Increases in cyclic GMP levels in brain and liver with sodium azide an activator of guanylate cyclase

SODIUM azide produces extrapyramidal symptoms with necrosis of the cerebral cortex, cerebellum, and basal ganglia. Toxicity with this metabolic inhibitor may also include hypotension, blindness and hepatic necrosis^{1–5}. Many hormones and drugs mediate their effects by altering the intracellular levels of cyclic AMP and/or cyclic GMP^{6–10}. We found that NaN₃ increases cyclic GMP levels in incubations of slices from cerebral cortex, cerebellum or liver, and also stimulates guanylate cyclase activity. In contrast to other agents that can increase cyclic GMP accumulation in tissues the effect of NaN₃ is observed in the absence of added calcium.

In slices from cerebral cortex, cerebellum and liver cyclic GMP levels increased within 5 s after the addition of 1 mM NaN₃ and, in 1–5 min, reached maximal levels (two to fivefold increases) that were maintained for 10 min (Fig. 1). With 1 mM NaN₃ there was no change in the level of cyclic AMP in these tissues at any of the times (5 s–10 min) examined. The accumulation of cyclic GMP in cerebral cortex slices depended on the concentration of NaN₃. NaN₃ (0.15 mM) produced a half-maximal effect and 1 mM NaN₃ was maximally effective. NaCN, another metabolic inhibitor, had little or no effect on cyclic GMP or cyclic AMP levels in cerebral cortex incubations (Table 1). Atropine (0.01 mM), (±)-propranolol (0.01 mM),

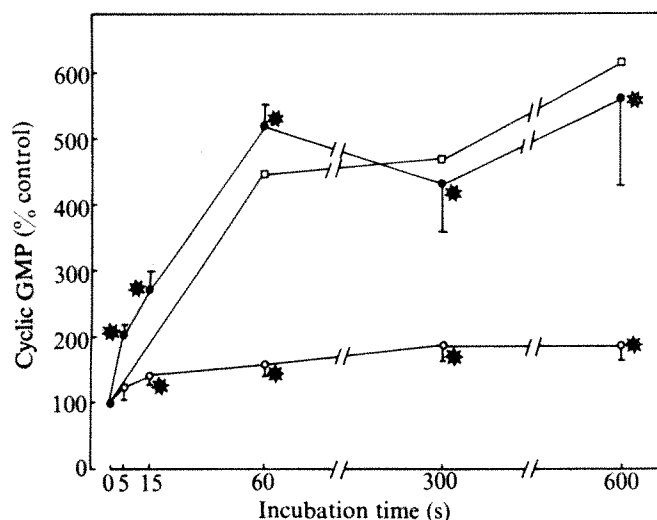


Fig. 1 Effect of NaN_3 on cyclic GMP levels in slices of cerebral cortex (●), cerebellum (○) and liver (□). Male Sprague-Dawley rats (150–200 g) were decapitated and slices of cerebral cortex, cerebellum and liver were incubated at 37 °C for 60–90 min in Krebs–Ringer bicarbonate (KRB) medium with 95% O_2 and 5% CO_2 . Slices were washed with fresh medium and incubated with 1 mM NaN_3 for the times indicated. Incubations were terminated by addition of 5% TCA and homogenised. Cyclic GMP and cyclic AMP were purified and assayed as described previously^{11–13}. Guanylate cyclase activity was determined as described previously¹³. Protein was estimated by the method of Lowry *et al.*¹⁴. Values presented are means from 2 to 10 incubations and are expressed as % control. Vertical bars indicate 1 s.e. Control values for cyclic GMP are 0.41 ± 0.09 ($n = 10$), 14.5 ± 2.5 ($n = 10$) and 0.06 ($n = 2$) pmol per mg protein for cerebral cortex, cerebellum and liver, respectively. * $P < 0.05$.

phenoxybenzamine (0.012 mM), diphenhydramine (0.1 mM), and γ -aminobutyric acid (1 mM) had no effect on cyclic GMP or cyclic AMP levels in cerebral cortex or cerebellum incubations with or without 1 mM NaN_3 . Hydroxylamine (0.1 mM), an agent that increases the concentration of γ -aminobutyric acid in brain⁶, markedly increased cyclic GMP levels in slices of cerebral cortex (Table 1). Aminooxyacetic acid (1 mM) which increases γ -aminobutyric acid levels in brain⁶, had no effect, however, on cyclic GMP or cyclic AMP levels in incubations of cerebral cortex slices with or without 1 mM NaN_3 .

The stimulatory effects of NaN_3 and NH_2OH on cyclic GMP accumulation were additive (Table 1).

The accumulation of cyclic GMP in incubations of cerebral cortex slices with 1 mM NaN_3 was unaltered when calcium-free medium was used (Table 1). The increase in cyclic GMP levels with 80 mM KCl, a depolarising agent at this concentration, required the presence of Ca^{2+} . The presence or absence of 2.5 mM Ca^{2+} in the incubation medium had a small effect on basal cyclic GMP levels. These results indicate that there are at least two mechanisms to increase cyclic GMP levels in tissues—

Fig. 2 Effect of NaN_3 on soluble (○) and particulate (●) guanylate cyclase from cerebral cortex. Rat cerebral cortex was homogenised at 4 °C in 9 volumes 0.25 M sucrose containing 5 mM Tris-HCl buffer, pH 8.0, using a glass homogeniser with a Teflon pestle. Homogenates were filtered through gauze and centrifuged at 105,000g for 60 min to separate soluble and particulate fractions. Enzyme preparations (20–50 μg protein) were preincubated for 5 min at 37 °C in mixtures of 50 mM Tris-HCl buffer, pH 7.6, 10 mM theophylline, 15 mM creatine phosphate, 20 μg creatine phosphokinase (120–135 U mg^{-1}), with or without NaN_3 as indicated, in a final volume of 100 μl . Reactions were started by addition of 3 mM MnCl_2 and 1 mM GTP (final concentrations), incubated for 5 min and terminated by adding 0.9 ml 50 mM Na acetate buffer, pH 4.0, and heated for 3 min at 90 °C (ref. 13). Cyclic GMP formed was determined by radioimmunoassay. Values presented are means of duplicate determinations.

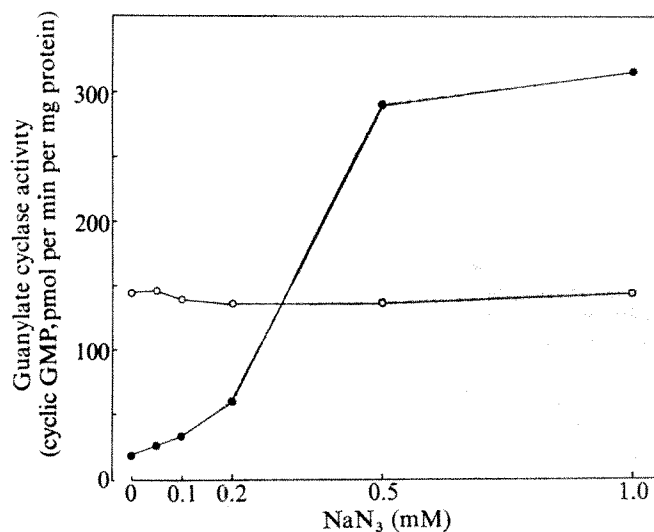


Table 1 Effects of NaN_3 and other compounds on cyclic nucleotide levels in slices of cerebral cortex in the presence and absence of Ca^{2+}

Addition	Incubation time (s)	Cyclic GMP (pmol per mg protein)		Cyclic AMP (pmol per mg protein)	
		KRB medium	Calcium-free medium	KRB medium	Calcium-free medium
None	—	0.77	—	2.85	—
NaCN (1 mM)	60	1.01	—	3.03	—
None	—	0.41	—	3.74	—
NH_2OH (0.1 mM)	60	2.74	—	2.88	—
NaN_3 (1 mM)	60	1.78	—	3.48	—
NH_2OH (0.1 mM) + NaN_3 (1 mM)	60	4.21	—	2.88	—
None	—	0.16	0.24	2.42	3.19
NaN_3 (1 mM)	15	0.44	0.85	2.72	3.23
NaN_3 (1 mM)	60	1.03	2.08	2.77	3.07
None	—	0.19	0.54	1.28	1.53
KCl (80 mM)	60	0.39	0.56	1.58	1.64
KCl (80 mM)	300	2.75	0.28	1.72	1.82

Cerebral cortex slices were incubated, washed with calcium-free KRB medium with 0.1 mM EGTA, and incubated an additional 30 min before NaN_3 or other agents were added. Slices were incubated with compounds indicated in the presence and absence of Ca^{2+} . Incubations were terminated by addition of 5% TCA and homogenised. Cyclic nucleotides were determined as described previously^{11–13}. When media containing no Ca^{2+} or high K^+ were used, the concentration of Na^+ was changed to maintain the total concentration of cation at 150 mM. Values presented are means of duplicate incubations.

one calcium-dependent and the other apparently calcium-independent.

The soluble and particulate guanylate cyclase activities in tissues have different properties and can be separated with gel filtration techniques¹³. Soluble guanylate cyclase demonstrates typical Michaelis-Menten kinetics and is activated by calcium. Particulate guanylate cyclase, which is associated with plasma membranes, Golgi and endoplasmic reticulum, is an allosteric enzyme and inhibited by calcium¹³. Soluble and particulate guanylate cyclase in cerebral cortex were examined, and we obtained results similar to those reported for other tissues. The particulate enzyme from cerebral cortex was markedly stimulated by NaN_3 , whereas NaN_3 had no effect on soluble guanylate cyclase (Fig. 2). The stimulation of particulate guanylate cyclase depended on the concentration of NaN_3 , with half-maximal activation occurring with 0.3 mM NaN_3 , a concentration similar to that required with slices. NH_2OH was a more potent stimulator of the particulate enzyme, but not the soluble enzyme from cerebral cortex. Half-maximal activation was obtained at 7 μM NH_2OH . Activation of guanylate cyclase by NaN_3 did not result from the preservation of substrate (GTP) or the inhibition of cyclic nucleotide phosphodiesterase activity. Various halide ions and cyanide (1 mM) had no effect on either soluble or particulate guanylate cyclase.

The presence of two forms of guanylate cyclase¹³ in tissues in which one is activated and the other is inhibited by Ca^{2+} may be the explanation for our observations. Generally the inhibitory effects of NaN_3 with a variety of enzymes require higher concentrations than those used in this study¹⁶. Cyanide, another metabolic inhibitor, had small effects on cyclic GMP levels and guanylate cyclase activity. The effects of NaN_3 and NH_2OH are probably not due to general inhibition of intracellular metabolism. Although the effects of NaN_3 on guanylate cyclase can explain the increased accumulation of cyclic GMP observed with intact tissues, other effects of NaN_3 cannot be excluded. NaN_3 and NH_2OH are known to be strong nucleophilic substances. Additional studies are also needed to determine whether or not the toxic manifestations of NaN_3 are related to the stimulation of guanylate cyclase and increased cyclic GMP accumulation in tissues.

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Positive control of cyclic AMP on mesenchymal factor controlled DNA synthesis in embryonic pancreas

THERE is increasing evidence that cyclic AMP and/or cyclic GMP are involved in the regulation of a number of metabolic processes including cell proliferation¹⁻¹³. We report here the results of an investigation testing the effects of cyclic AMP, cyclic GMP and the mesenchymal factor (MF)¹⁴⁻¹⁷ on the stimulation of DNA synthesis in embryonic epithelia in organ culture.

The normal development of most organ systems requires the interaction of epithelial and mesenchymal cells^{18,19}. We have used the pancreas as a paradigm for the epithelial-mesenchymal interaction. A factor obtained from mesenchymal tissues or from mesenchyme-rich embryonic tissues can substitute for the mesenchyme to induce normal growth and development¹⁴⁻¹⁷. MF activity is sensitive to both trypsin¹⁵ and periodate oxidation (ref. 17, and S. Levine, R. P. and W. J. R., unpublished observations), suggesting that protein and carbohydrate moieties are required for its function. Purified MF covalently bound to Sepharose beads induces DNA synthesis in the pancreatic epithelial cells. The experimental results suggest that MF functions through an interaction with the cell surface^{16,17}. The effects of MF may therefore be mediated by secondary messengers such as cyclic AMP or cyclic GMP. We have therefore tested the effects of these compounds as well as

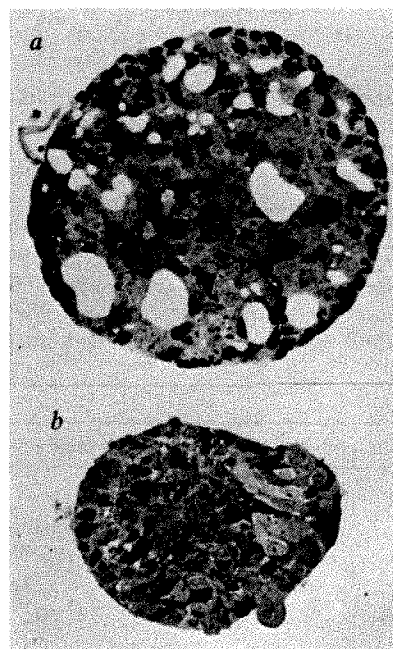


Fig. 1 The incorporation of ^3H -thymidine in the presence of inactivated MF and cyclic AMP is due to the labelling of many cells. Pancreatic epithelia were isolated and cultured as described in Table 1 in medium containing 1 mg ml⁻¹ of inactivated factor in the presence (a) or absence (b) of 10^{-3} M 8-OH-cyclic AMP. The epithelia were harvested and fixed in 4% glutaraldehyde in 0.04 M phosphate buffer (pH 7.4). After postfixation in osmium they were embedded in Epon²⁰. Thick sections were placed on the slides and coated with an Ilford L-4 emulsion²⁰ and developed after 10 d of exposure. After 90 min of treatment with sodium periodate MF is not totally deprived of activity: by increasing its concentration there is higher thymidine incorporation (see Fig. 2b) than is reflected by a few labelled cells in Fig. 1b. But the addition of 8-OH-cyclic AMP increases dramatically the number of cells which undergo DNA synthesis (Fig. 1a). The vacuoles observed are caused by the addition of cyclic AMP and probably reflect the increased fluid transport into the existing exocrine lumen which is already present at that time of development²¹.

their derivatives on the DNA synthesis of pancreatic epithelia (mesenchyme-free) in the presence and absence of MF. None of the compounds affected MF activity; but unexpectedly, MF which had been inactivated could be completely reactivated by inclusion of dibutyryl cyclic AMP (db-cyclic AMP) or 8-OH-cyclic AMP though not by cyclic AMP, cyclic GMP or cyclic GMP analogues. The effects of 8-OH-cyclic AMP and 8-Br-cyclic GMP on DNA synthetic activity in the presence of MF or inactivated MF are summarised in Table 1. Neither 8-OH-cyclic AMP nor 8-Br-cyclic GMP stimulated or inhibited DNA synthesis when the epithelia were incubated in the presence of active MF. On the other hand, 8-OH-cyclic AMP but not 8-Br-cyclic GMP stimulated the activity of periodate-inactivated MF to the levels of incorporation observed with untreated MF. We have shown previously that in the presence of MF, ^3H -thymidine is incorporated into the DNA in many cells, whereas, in contrast, incubation with inactivated MF leads to ^3H -thymidine incorporation in very few cells¹⁷. Figure 1a and b demonstrates that the addition of 8-OH-cyclic AMP increases thymidine incorporation into cells in the presence of inactivated MF. Thus the effect of cyclic AMP is to increase the proportion of cells undergoing DNA synthesis. The specificity of this response is reported in Table 2. Whereas 8-OH-cyclic AMP and db-cyclic AMP stimulate DNA synthesis in the presence of inactivated MF, neither 5'-AMP nor cyclic AMP itself even when present with the phosphodiesterase inhibitor, caffeine, promote DNA synthesis. Some MF preparations that become inactive spontaneously can also be activated by db-cyclic AMP derivatives. The activation of DNA synthesis by db-cyclic AMP or 8-OH-cyclic AMP but not cyclic AMP is taken to mean that entrance into the cells is a requisite for activity. All attempts to reactivate the factor itself by treatment with cyclic AMP or cyclic AMP analogues before its addition to the cells have failed. For example, inactivated MF incubated for 20 h at 37 °C with 10^{-3} M db-cyclic AMP is inactive after removal of the free cyclic nucleotides by chromatography on Sephadex G-25, but stimulates DNA synthesis on addition of db-cyclic AMP into the culture medium. The incorporation of ^3H -thymidine into DNA is dependent on both the concentration of inactivated MF and 8-OH-cyclic AMP. As shown in Fig. 2a, increasing the concentration of inactive factor in the presence of 10^{-3} M 8-OH-cyclic AMP results in an increase of thymidine incorporation into DNA. In fact, the saturation profile is similar to that observed with active MF alone¹⁷. In the presence of a single concentration of MF (Fig. 2b), addition of 8-OH-cyclic AMP gives a typical hyperbolic activation profile (low levels of activity are always found with periodate inactivated MF preparations). The activation

Table 1 Inactivated MF stimulates DNA synthesis in the presence of cyclic AMP but not cyclic GMP

	8-OH-cyclic AMP (10^{-3} M)	8-Br-cyclic GMP (10^{-4} M)	^3H -Thymidine incorporated into DNA (c.p.m. per epithelium per 6 h)	<i>n</i>
Active MF	—	—	7,330 (5,200/9,800)	8
	+	—	8,680 (5,660/13,100)	6
	—	+	7,495 (6,700/10,000)	10
Inactivated MF	—	—	290 (97/420)	6
	+	—	6,500 (3,300/8,020)	8
	—	+	264 (180/405)	10

Inactivated MF stimulates DNA synthesis in the presence of cyclic AMP but not cyclic GMP. Pancreatic epithelia were isolated from rat embryos on day 12 of gestation (about 35 somites) as previously described¹⁴⁻¹⁷. Individual epithelia were transferred in Dispo tray wells (Linbro, Los Angeles) and cultured for 18 h in 100 μl of medium (Eagle's basal medium in which the essential amino acids have been increased sevenfold ($7 \times \text{BME}$), plus 1 mg ml^{-1} bovine serum albumin, 1 μg , ml^{-1} penicillin, 10^{-4} mg ml^{-1} streptomycin and 25 μg ml^{-1} fungisone) in an atmosphere of 5% CO_2 in air. The nucleotides were dissolved in the $7 \times \text{BME}$; and 100 μl of this solution was added per ml of culture medium. Dilutions were made from a 10^{-3} M stock solution which was kept frozen at -70°C . Inactivated MF or MF was present at a final concentration of 600 μg protein ml^{-1} . For assays of DNA synthesis the epithelia were further incubated for 6 h in fresh identical culture medium containing ^3H -thymidine (10 μCi ml^{-1}). At the end of this period the epithelia were harvested, washed three times with EBSS containing 0.1% albumin, transferred into microfuge tubes (Beckman) and frozen. To measure the thymidine incorporated 100 μl 0.1% SDS was added and the tissue was disrupted by sonication at 0°C by rubbing the probe of the sonicator along the tube for 30 s. Aliquots (40 μl) of the suspension were spotted on GF Whatman filters. The filters were washed 6 times with cold 5% TCA, 3 times with 95% ethanol and twice with ether. The filters were dried and counted in scintillation fluid (100 ml NCS, 16 ml H_2O , 15.3 g Omnifluor added to 3.8 l of toluene). Results are expressed as c.p.m. ^3H -incorporated per epithelium during the 6-h incubation in the presence of ^3H -thymidine. MF was prepared as described¹⁶. For oxidation with sodium periodate, MF in 0.8 M KCl, 0.01 M Tris-HCl buffer (pH 8.0) was dialysed against 0.1 M glycine buffer at pH 8.0. After being dissolved in the same buffer sodium periodate was added at a final concentration of 50 mM. The reaction was allowed to proceed at 0°C in the dark for 90 min. To stop the oxidation tenfold molar excess of glycerol was added. After 15 min the periodate-treated MF (IO_4MF) was dialysed against 0.1 M glycine buffer (pH 8) followed by dialysis against 0.1 M glycine (pH 9). The preparation was then treated with sodium borohydride, dissolved in 0.1 M NaOH and added at a final concentration of 0.1 M. The preparation ($\text{IO}_4\text{BH}_4\text{MF}$) was dialysed against 0.01 M glycine buffer (pH 9). This fraction is referred to as inactivated MF. Identical results have been obtained if IO_4MF is not reduced with sodium borohydride. The effect of the oxidation seems specific: control experiments (S. Levine, R.P., and W.J.R., unpublished) show the sodium periodate is not toxic by itself since simultaneous addition of IO_4Na and glycerol have no effect on MF activity no more than direct reduction of MF by NaBH_4 . The inactivation by oxidation does not result from activation of an inhibitor since mixing of MF and IO_4MF does not decrease the activity of MF. The numbers in parentheses represent the lowest and highest values. *n* indicates the number of epithelia assayed.

Fig. 2 The degree of stimulation of DNA synthesis is dependent on the concentration of both inactivated MF and the cyclic AMP derivative. Pancreatic epithelia were isolated, cultured and DNA synthesis measured as described in Table 1. a, 9 epithelia were cultured in the presence of various concentrations of inactivated factor in the presence (●) or absence (○) of 10^{-3} M 8-OH-cyclic AMP. Periodate borohydride treated MF was concentrated up to 14 mg ml^{-1} in an Amicon stirred cells (Amicon Corp., Lexington, Massachusetts) using a PM-10 filter. The intermediate concentrations were obtained by dilution from that stock solution and added as usual at 100 μl ml^{-1} . The increase of thymidine incorporation in epithelia cultured in the presence of concentrated inactivated MF is due to the fact that after 90 min oxidation there is some remaining activity. b, Sets of 9 epithelia were cultured in the presence of 300 μg ml^{-1} of inactivated MF and various concentrations of 8-OH-cyclic AMP. The bars represent the s.e.m. of the tritium counts.

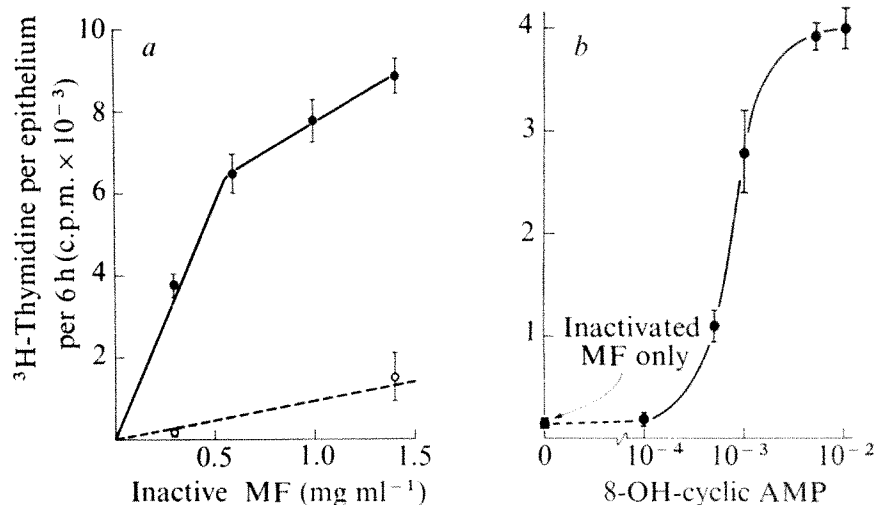


Table 2 DNA synthetic activity in the presence of inactive MF is dependent on cyclic AMP

	Nucleotides (10^{-3} M)	^3H -Thymidine incorporation into DNA (c.p.m. per epithelium per 6 h)	<i>n</i>
Inactivated MF	—	160 (128/190)	9
Inactivated MF	db-cyclic AMP	3,009 (870/5,630)	8
Inactivated MF	8-OH-cyclic AMP	3,370 (2,230/4,040)	9
Inactivated MF	Cyclic AMP	139 (110/190)	9
Inactivated MF	Cyclic AMP + caffeine (10^{-2} M)	267 (91/380)	9
Inactivated MF	5'-AMP	139 (115/390)	9
Inactive MF	—	205 (116/392)	5
Inactive MF	db-cyclic AMP	9,361 (7,625/10,795)	6

DNA synthetic activity in the presence of inactive MF is cyclic AMP dependent. Experiments were carried out as described in the legend of Table 1. Inactivated MF was present at a concentration in the incubation medium of $300 \mu\text{g ml}^{-1}$ and inactive MF at $600 \mu\text{g ml}^{-1}$. Inactive MF refers to MF preparations which spontaneously lose activity upon storage at 0°C . In such conditions, MF may lose its activity in a few days, but such inactive MF as well as IO_4MF or $\text{IO}_4\text{BH}_4\text{MF}$ are reactivated by cyclic AMP derivatives even after weeks of storage at 0°C . The numbers in parentheses represent the lowest and highest values. *n* represents the number of epithelia assayed

profile of db-cyclic AMP is different: a peak of activity is reached between 5×10^{-3} and 10^{-3} M. At higher concentrations inhibition is observed, perhaps because of the accumulation of toxic levels of butyrate.

As shown in Table 3, inactive MF is required for the activation of DNA synthesis. Cyclic GMP or cyclic GMP derivatives do not stimulate DNA synthesis by themselves. Furthermore, addition of varying concentrations of 8-Br-cyclic GMP in the presence of optimal levels of 8-OH-cyclic AMP do not induce DNA synthesis in the absence of inactivated MF (Table 3). These experiments give no evidence for a role of cyclic GMP as an intermediate in the action of MF.

Several processes that are dependent on cyclic AMP have been shown to require or involve calcium²⁰. In these systems calcium ionophores frequently influence the process²¹⁻²⁴. We have therefore investigated the effect of the calcium ionophore A23187 on DNA synthesis in the pancreatic epithelia. This compound at levels between 10^{-7} and 10^{-6} M does not stimulate the incorporation of ^3H -thymidine into DNA. In fact, at the highest concentrations tested (4×10^{-7} and 10^{-6} M) there is inhibition of the usual stimulation produced by inactive MF and 8-OH-cyclic AMP. We consider the experiments with calcium ionophore equivocal because these compounds allow transport of other ions and are present over relatively long periods of time (24 h) during which the stimulation of incorporation of precursors into DNA is observed.

The results of the present investigations suggest that our mesenchymal factor preparations contain two functional activities. This may explain our previous difficulties in

purification of MF and suggests that assays in the presence of 8-OH-cyclic AMP may detect one component, and that assays in the presence of inactive factor detect another function. The results of the present experiments suggest that one function, perhaps requiring a carbohydrate moiety (because of the sensitivity to periodate oxidation), may act through the enhancement of intracellular levels of cyclic AMP. The evidence for this is admittedly indirect, since intracellular levels of cyclic AMP have not been measured. The specificity of the response for cyclic AMP derivatives which penetrate cells and the magnitude of the response are, however, compatible with this view. The mode of action of cyclic AMP in this system is not yet resolved and an indirect effect of cyclic AMP is possible: intracellular cyclic AMP may enhance the responsiveness of the cell to 'inactive factor' by, for example, increasing the density of appropriate receptors on the cell membrane.

It is somewhat unexpected that the cyclic AMP analogues have a positive effect on DNA synthesis. In other experiments involving cells in culture, addition of compounds which increase intracellular cyclic AMP levels inhibit DNA synthesis¹⁻⁴. Furthermore, in a variety of cells there seems to be an inverse correlation between the level of intracellular cyclic AMP and DNA synthesis⁶⁻⁹. There seems also to be a positive correlation between increased cyclic GMP levels and DNA synthesis in some systems¹⁰⁻¹³. These results contrast with the findings reported here. Cyclic AMP is required for DNA synthesis; cyclic GMP or its derivatives have no effect by themselves, and do not inhibit the cyclic AMP response. It is possible that secretory cells whose secretory functions are positively regulated by cyclic AMP may also develop under the positive control of cyclic AMP. The positive control by cyclic AMP is analogous to that inferred in the action of a number of other peptide hormones²⁵⁻²⁸ which apparently act through cyclic AMP. The response observed in the embryonic pancreatic cells may also represent a characteristic of differentiating systems, that is, that high levels of cyclic AMP are required for a differentiative transition. Thus, proliferation of cells undergoing differentiation may have a different regulatory mechanism than the various cell types normally studied in cell culture. The developing pancreatic epithelium may be useful to define the role of cyclic AMP on the regulation of DNA synthesis and cytodifferentiation.

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Table 3 Addition of cyclic GMP does not influence DNA synthesis in embryonic pancreatic cells

Protein added to medium	Addition					
	None	8-OH-cyclic AMP (10^{-3} M) + 8-Br-cyclic GMP (M)				
		—	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Inactivated MF	205 (140/330)	1,414 (751/2,112)	2,025 (1,540/2,500)	2,098 (1,230/2,420)	1,978 (650/2,980)	2,452 (1,630/3,100)
Albumin	144 (106/195)	162 (91/233)	178 (71/362)	213 (143/501)	247 (107/552)	303 (210/403)

Addition of cyclic GMP or its derivatives does not influence DNA synthesis of embryonic pancreatic cells. Experiments were carried out in the same way and conditions are as described in the legend for Table 1. Inactivated MF was added at a concentration of about $300 \mu\text{g ml}^{-1}$ and albumin at 1 mg ml^{-1} . The addition of db-cyclic GMP or cyclic GMP in place of 8-Br-cyclic GMP led to identical results. The numbers are the average of 8 or 9 epithelia; the numbers in parentheses correspond to the lowest and highest values.

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Ultraviolet light-induced unscheduled DNA synthesis in mouse oocytes during meiotic maturation

ULTRAVIOLET light-induced incorporation of ^3H -thymidine into DNA by non-S-phase nuclei has been observed in cultured mammalian cells¹, spermatogenic cells²⁻⁴, and embryos⁵. This unscheduled DNA synthesis is generally considered to be the result of excision and replacement of ultraviolet-damaged bases by repair enzymes⁶⁻⁸. We have examined the repair capacity of full-grown mouse oocytes after exposure to ultraviolet irradiation. As sensitivity to chemical mutagens and X irradiation changes during meiotic maturation in many species, we determined whether the ability to repair damage to DNA changes during mouse oocyte maturation *in vitro*. Oocytes were found to be capable of unscheduled DNA synthesis during meiotic maturation; however, irradiation of germinal vesicle stage oocytes induced higher levels of ^3H -thymidine incorporation than irradiation at metaphase I or metaphase II stages.

Fully-grown oocytes aseptically isolated from the ovary of adult mice were cultured in chemically defined medium⁹. Oocytes were irradiated 2-18 h after isolation using germicidal lamps, and incubated immediately in medium containing ^3H -thymidine. Non-irradiated oocytes served as controls. Incorporation of ^3H -thymidine into chromosomes was studied by autoradiography.

The progression of meiotic maturation of the oocytes during the culture period is summarised in Table 1. Many of the oocytes underwent germinal vesicle (GV) breakdown within 3 h of isolation. Metaphase I (MI) was reached by 4-8 h after the start of culture. Chromosomes at this stage were short and thick and were usually recognised as pairs of homologues. Oocytes cultured for 16-18 h reached second metaphase (MII), as evidenced by appearance of the first polar body (PBI). Oocyte chromosomes at MII were more clustered than those at MI. Whereas the morphology of the MII chromosomes was

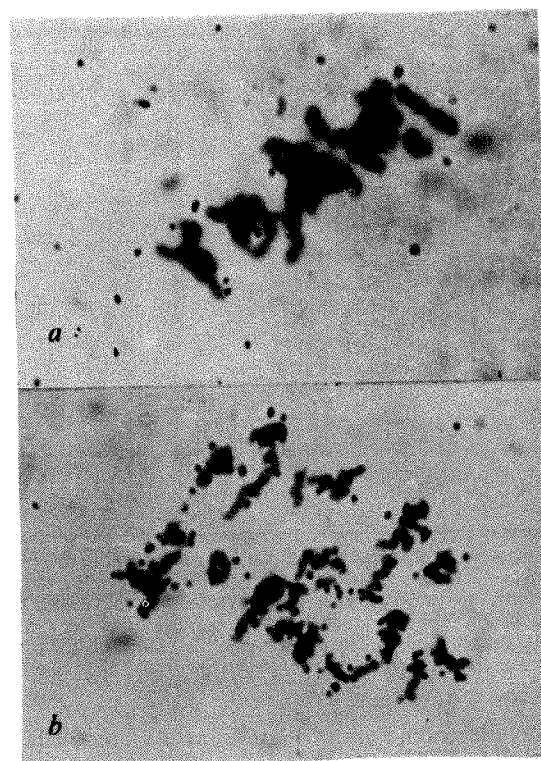


Fig. 1 Unscheduled DNA synthesis in mouse oocytes. *a*, MII control ($\times 1,150$). *b*, MII irradiated (60 J m^{-2} ; $\times 800$). Oocytes were irradiated in isotonic PBS containing 2 mg ml^{-1} polyvinyl pyrrolidone at 2-18 h after isolation using germicidal low pressure mercury discharge lamps (dose rate $1.3 \text{ J m}^{-2} \text{ s}^{-1}$) and immediately incubated in medium containing ^3H -thymidine ($20 \mu\text{Ci ml}^{-1}$; specific activity 40 Ci mmol^{-1}) for 1 h and cultured for 1 h in unlabelled medium. Non-irradiated oocytes served as controls. After washing, all oocytes were fixed (ethyl alcohol-glacial acetic acid, 3:1), placed on microscope slides, and incubated at 37°C in an atmosphere of 45% acetic acid for 5-10 min and air dried. Before dipping the slides in Kodak NTB emulsion, they were soaked in 5% trichloroacetic acid at 4°C for 30 min, washed in 95% ethanol and air dried. Exposure to the emulsion was for 2 weeks.

well preserved (Fig. 1*a*), PBI chromosomes deteriorated, with bridge formation and fusion, into a mass of chromatin.

Autoradiography showed that grain counts over the GV or chromosome set were significantly higher (Student's *t* test, $P < 0.001$) after ultraviolet irradiation than in controls, regardless of the stage of meiotic maturation at which they were irradiated (Table 2, Fig. 1*b*). The corrected oocyte grain count increased with dose at 30 and 60 J m^{-2} but, except for the MI oocyte, there was no further increase in grain count at 120 J m^{-2} . Irradiated PBI had significantly higher observed and corrected grain counts than controls at a dose of 30 J m^{-2} ($P < 0.001$), approximately half that localised over MI or MII chromosomes; but corrected grain counts for the PBI were not significantly above controls when higher doses were given, suggesting that the slight repair capacity of the PBI was lost, perhaps by ultraviolet disruption of polar body integrity.

Grain counts over ultraviolet-irradiated oocytes differed

Table 1 Progression of meiotic maturation in mouse oocytes in culture

Time in culture (h)	No. of oocytes	% Oocytes		
		GV	MI	MI
2-4	108	33	67	0
6-8	12	0	100	0
16-18	120	7	15	78

Fully grown oocytes were isolated aseptically from the ovaries of 8-10-week-old mice (DUB/ICR strain, Flow, Dublin, Virginia) and cultured at 37°C with an atmosphere of 5% CO_2 -95% air in oocyte culture medium⁹.

Table 2 Incorporation of ^3H -thymidine into chromosomes of oocytes

Stage of oocytes	Dose (J m^{-2})	No. of oocytes	Grain counts on GV and chromosomes	
			Observed grains (mean \pm s.e.)	Corrected for background grains (mean \pm s.e.)
GV	0	13	19.6 \pm 7.0	3.8 \pm 6.0
	30	4	182.3 \pm 90.7	126.8 \pm 80.6
	60	13	694.5 \pm 133.0	605.4 \pm 114.6
	120	16	600.9 \pm 155.8	576.7 \pm 149.0
MI	0	23	2.2 \pm 0.8	-4.2 \pm 1.9
	30	8	34.1 \pm 8.9	37.2 \pm 11.7
	60	32	65.4 \pm 10.1	76.7 \pm 11.2
	120	38	83.9 \pm 10.7	89.6 \pm 12.7
MII	0	16	4.9 \pm 2.4	3.2 \pm 1.8
	30	11	30.0 \pm 5.5	29.7 \pm 5.8
	60	11	43.9 \pm 9.9	37.0 \pm 11.3
	120	15	39.3 \pm 6.5	39.5 \pm 7.4
PBI	0	21	2.5 \pm 0.5	-2.2 \pm 2.1
	30	12	18.8 \pm 6.7	15.8 \pm 7.4
	60	49	10.3 \pm 1.7	9.7 \pm 2.2
	120	12	11.4 \pm 3.3	7.6 \pm 4.0

Oocytes were treated as in Fig. 1. Grains on autoradiographs were counted using an ocular micrometer sectioned in $100 \mu\text{m}^2$ areas. Number of grains observed over chromosomes or GV was scored. As the grain density of background as well as the area of the chromosomes or GV varied, however, grain number was corrected for background by the following formula: corrected grain counts = $(A - N)(B/n)$, where N is the number of unit areas in which all chromosomes or GV is confined and A is the total grain number in N areas, n is the number of background unit areas adjacent to the chromosomes or GV, and B is the total grain count in n areas. This correction gave negative values for control oocytes when grain counts over chromosomes were lower than the average background counts.

markedly depending on the stage of meiotic maturation, whereas those of control oocytes invariably remained low (Table 2, Fig. 2). There was a large number of grains on GVs of irradiated oocytes, whereas fewer grains were localised over MI and MII chromosomes, and fewer still over polar body chromosomes. Because of the intervening meiotic reduction division, grain count over a chromosome set at MII should be half that over MI chromosomes, assuming that ultraviolet penetration and tritium self-absorption is the same in both situations. At a dose of 30 J m^{-2} , corrected grain counts over MI and MII chromosomes were not significantly different ($P \approx 0.2$), whereas at 60 and 120 J m^{-2} , MI grain counts were approximately twice those of MII chromosomes. These results suggest that repair capacities are similar at these two stages, although considerably lower than in the intact GV. Because of the high grain counts over GV-2 stage oocytes, compared with later stages, we determined whether there was a decrease in thymidine uptake during oocyte maturation. Samples of 10 oocytes were incubated in $20 \mu\text{Ci ml}^{-1}$ ^3H -thymidine for 1.5 h, washed in isotonic phosphate buffered saline (PBS) and collected on $5 \mu\text{m}$ Millipore filters by suction filtration. Cells were dissolved in 1% Triton X-100 and samples were counted with a liquid scintillation spectrometer. We found that there was no significant difference in uptake between the GV stage (44.2 c.p.m. per egg, three samples) and the MII stage (52.3 c.p.m. per egg, four samples). Thus, it seems that the higher GV grain counts are due to greater levels of thymidine incorporation into DNA, although higher grain counts could result in part from higher autoradiographic efficiency for the flattened GV nuclei than for condensed chromosomes.

In these experiments mouse oocytes exhibit unscheduled DNA synthesis after ultraviolet irradiation, and thus are capable of excision repair of DNA damage at these stages. Spermatogonia and spermatocytes of several mammalian species also exhibit unscheduled DNA synthesis after ultraviolet irradiation²⁻⁴ or exposure to a chemical mutagen, ethyl methane sulphonate¹⁰. In contrast, spermatids and spermatozoa show little or no unscheduled DNA synthesis with these treatments^{2-4,10}. As the excision repair system seems to act on a variety of base alterations¹¹, it may confer some protection against environmental base damage in mammalian germ cells during meiotic maturation.

The dose rate effects for X-ray-induced mutations of mouse oocytes have been interpreted as indicating the presence of an X-ray repair system during oogenesis^{12,13}. Oocyte radio-

sensitivity follows the same pattern in diverse animals, including mice¹⁴⁻¹⁶. During meiotic maturation, mouse oocytes are the least sensitive to X-ray induction of mutations or chromosome aberrations before GV breakdown, then become more sensitive at MI (ref. 17). Although repair of ultraviolet and X-ray damage to DNA involves several distinct processes, the base-damage component of X-ray damage could probably be repaired during meiotic maturation stages of oocytes, and changes in excision repair capability may account in part for the known changes in X-ray sensitivity. In addition to providing protection from genetic damage at meiotic maturation stages, the excision repair capacity seen here may contribute to the repair capabilities that have been observed in early stages of mouse embryos⁵.

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Duplex microtubule is a new form of tubulin assembly induced by polycations

INTACT microtubules surrounded by a coat of extra material and stabilised by polycations such as Alcian blue, have been seen by Behnke¹ in brain extracts. A similar structure was reported in preparations of microtubules reassembled *in vitro* when DEAE-dextran was added (ref. 2) and the extra material was attributed to DEAE-dextran itself. In this note and the accompanying communication by Behnke³ evidence is presented that the outer component is an additional shell of tubulin subunits probably complexed with polycations. This structure is related to a double ring form of tubulin, which is a putative nucleating centre for microtubule assembly.

Highly purified tubulin prepared from pig brain⁴ was polymerised at 37 °C to form microtubules. Protamine was added at a twofold molar excess over protein, and there was an immediate increase in turbidity. The microtubules, which had the characteristic 40 Å axial periodicity and 250 Å diameter, were surrounded along their length by an outer wall which had an overall diameter of 380 Å as Fig. 1 shows. Apart from some protamine, protein estimation and sodium dodecyl sulphate (SDS) gel electrophoresis show that the protamine-treated tubules consist of tubulin (97%) and the high molecular weight components known to be associated with microtubules². Too little non-protein material is present (< 10%) to account for the outer wall. Since this structure is formed with such a variety of polycations^{2,3} and consists almost entirely of tubulin, it is a new tubulin assembly which we propose to call a duplex microtubule.

When protamine at less than equimolar concentration with respect to protein is added, regions of both duplex and microtubule are seen along any one length of tubule (Fig. 1a). The protofilamentous appearance of the microtubule is also visible at the centre of the duplex, whereas the predominant feature of the outer wall, seen clearly at the edges of the complex, is an axial repeat of 50 Å. Optical diffraction patterns of the duplex structure (Fig. 2) are consistent with these observations. Weak but clear off-meridional reflections corresponding to an axial spacing of 40 Å are seen which are characteristic of patterns from axonemal⁵ and reconstituted⁶ microtubules. Also there are strong off-meridional reflections at $1/50 \text{ Å}^{-1}$ which we have associated with a helical arrangement of subunits in the outer wall. The whole structure contributes to the equatorial pattern and for this reason the other reflection characteristic of microtubules, namely an equatorial at 50 Å (refs 5 and 6) has not been clearly identified. The pattern also shows other reflections; these and the ones we have described indicate that the duplex structure is complex. A simpler structure which resembles the outer wall by itself is, however, obtained by adding spermine to tubulin at 4 °C. (Fig. 1c). Its diameter is similar to that of the duplex tubule and the 50-Å axial periodicity can be seen. Its diffraction pattern (not shown) shows the strong 50-Å off-meridional spots and in addition strong near equatorial reflections at a spacing corresponding to 40 Å. The dimensions of this lattice (50 Å axially \times 40 Å azimuthally) are therefore the same as the size of the subunits in a microtubule (40 \times 50). This implies that the subunits in both the inner and outer wall of the duplex tubule are the same but oriented perpendicular to each other in the two layers of the complex.

The structure of the duplex microtubule was investigated further by comparing its stability with that of microtubules at 4 °C. The ring forms produced on cooling are summarised in Table 1. Microtubules, in the absence of protamine, are completely dissociated after 30 min at 4 °C into single rings of outer diameter 380 Å, inner diameter

250 Å (30–36S protein)^{2,7} and particles, possibly dimers (6S protein)^{2,7}. These single rings have the same outer diameter as the duplex tubules and are approximately the same as the outer diameter of the single rings, double rings and helices reported previously for similar conditions^{2,8}.

In the presence of protamine, duplex and microtubules are seen even after 1 h at 4 °C as well as single rings and double rings (Fig. 1b). Both these rings have the same outside diameter as the duplex microtubules (Table 1) as seen fortuitously in Fig. 1b where a ring is superimposed on a tubule. Double rings together with short segments of duplex

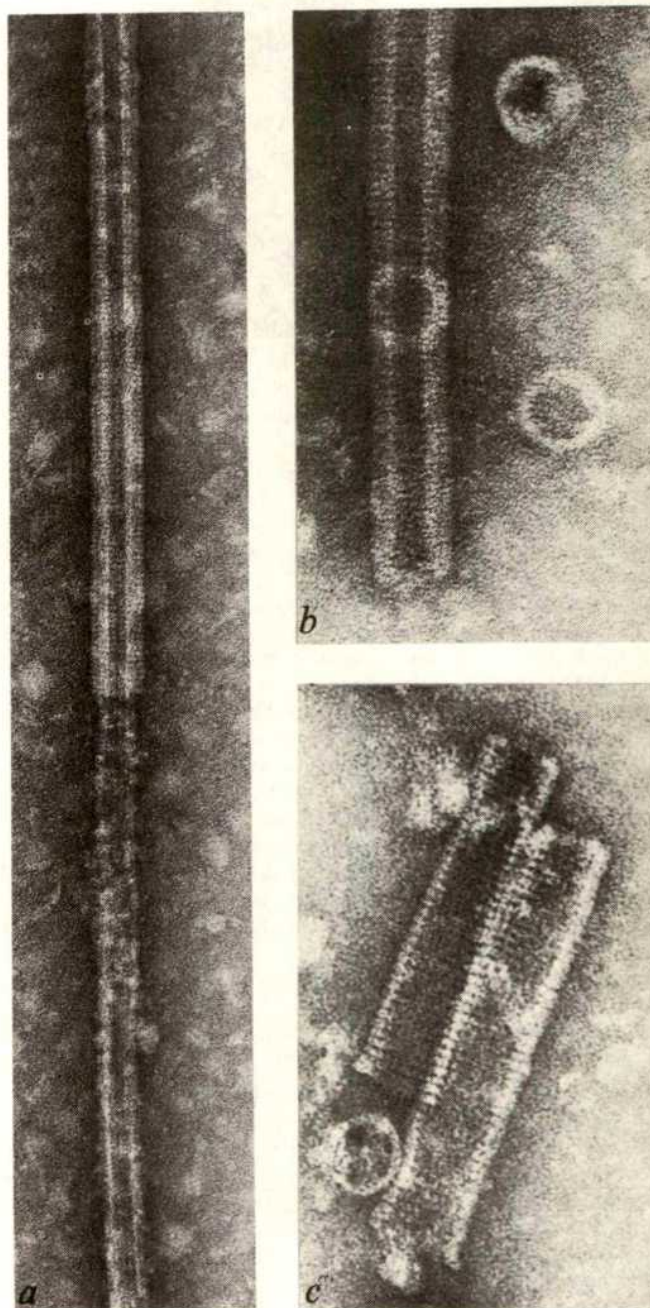


Fig. 1 Polymorphic forms of tubulin. *a*, Regions of duplex microtubule induced by low levels of protamine. Microtubules from purified tubulin⁴ (10 μM) in reassembly buffer (100 mM MES, 1 mM EGTA, 0.5 mM Mg^{2+} , 1 mM GTP, pH 6.8) were incubated with neutralised protamine (5 μM). ($\times 150,000$.) *b*, Duplex tubules cooled at 4 °C for 1 h. The preparation shows both undissociated duplex tubules and ring forms. The fortuitous superposition of the single ring on the duplex tubule illustrates that their diameters are the same. ($\times 250,000$.) *c*, Spermine-induced tubule formation. Purified tubulin (1.8 μM) was incubated at 4 °C with neutralised spermine (1.5 μM), giving rise to a structure resembling the outer wall of the duplex structure. ($\times 250,000$.) Samples for electron microscopy were negatively stained with 2% uranyl acetate.

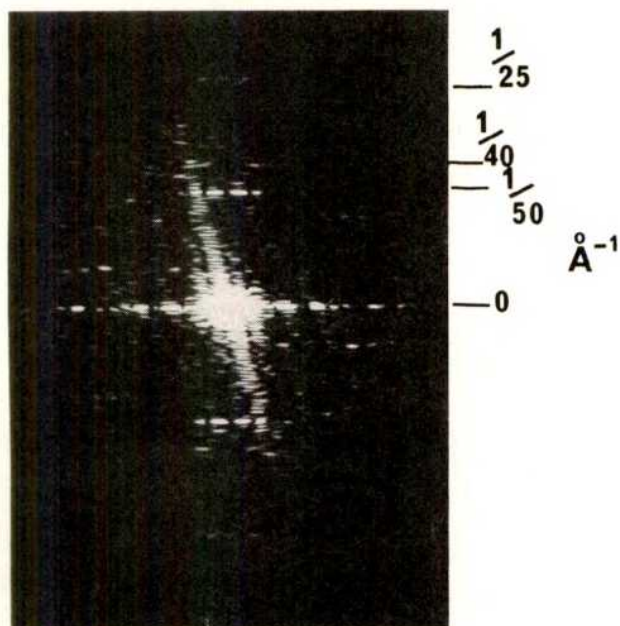


Fig. 2 Optical diffraction pattern of a duplex tubule showing 40-Å microtubule reflections and the 50-Å off-meridional reflections from the outer wall. The 50-Å reflections are also seen in patterns from spermine induced tubules.

again with diameter 380 Å were formed by adding protamine to tubulin at 4 °C, (Fig. 3). These rings are so well preserved that often 13 subunits (the number of protofilaments in a microtubule) can be counted in the inner ring (Fig. 3, inset). Significantly the outer diameter of this inner ring is 250 Å, the same as the microtubule. The double ring therefore has the same cross section as the duplex (Table 1). The subunits in the outer ring cannot be counted, suggesting that the molecules are differently arranged.

Some of the structures induced by polycations may also be formed *in vivo*. The duplex microtubule is seen occasionally in brain extracted with a special microtubule stabilising medium¹. Macrotubules of diameter about 340 Å are formed in cells subjected to cold⁹, high pressure⁹ and drugs¹⁰. One form¹⁰, in particular, is found in the presence of vinblastine, itself a polycation. It is not, however, clear from these observations whether these structures are the same as the duplex tubule or the outer wall, as in the spermine-generated tubules.

The formation of the duplex tubule *in vitro* may provide clues to the mechanism of microtubule assembly which is at present unclear. Previous suggestions have involved the unrolling of rings into protofilaments^{7,8}, or the rearrangement of stacks of rings into a helix², but both pro-

posals require large subunit rearrangement. Another possibility follows from the observations presented here. Before tubule formation the dominant form, at least in our preparations, is the 380-Å diameter single ring (or short segment of helix). A ring form (although not necessarily the same one for each proponent) is generally agreed to be a necessary step in polymerisation and a factor which converts 6S protein to a ring form has been described by Weingarten *et al.*¹².

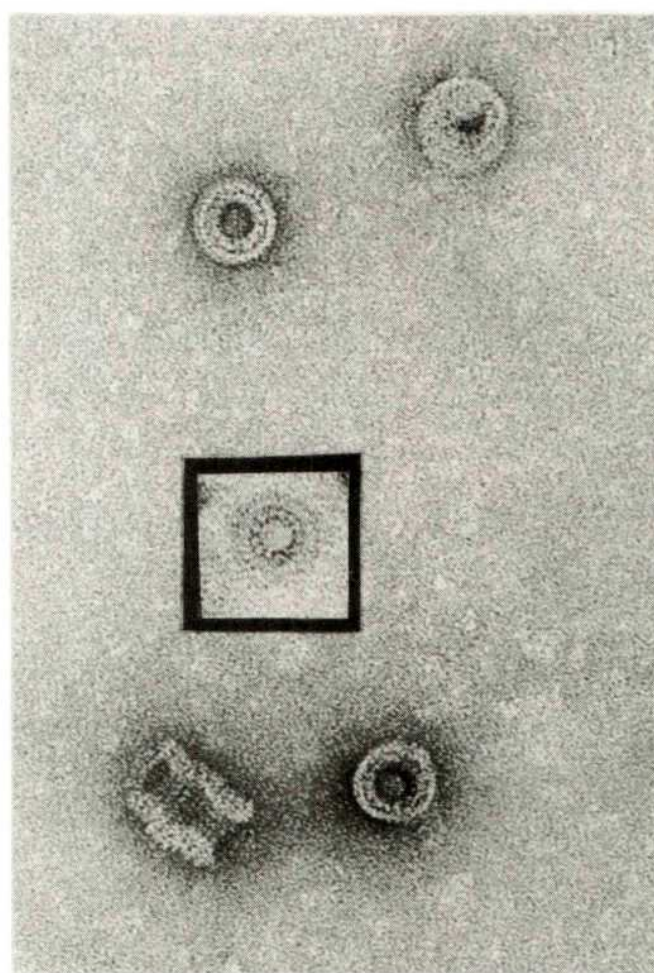


Fig. 3 Double rings and short segments of duplex tubule seen when protamine is added to tubulin at 4 °C. Insert shows double ring with 13 subunits in the inner ring. The samples were negatively stained with 2% uranyl acetate. ($\times 250,000$.)

We consider a plausible sequence of events is first the formation of the 380-Å diameter ring or segment of helix from 6S protein. This structure then serves as a template for the formation of a second ring or segment of helix on the inside. The double ring is stabilised by polycations but could occur naturally in small amounts and is possibly the disk first described by Borisy *et al.*¹¹. Polymerisation proceeds by growth of this structure to give a short segment of duplex tubule. This short segment then acts as a template for microtubule polymerisation, the microtubule growing from the inner ring. In support of this model, it has been shown that 6S tubulin which does not normally polymerise by itself, will assemble onto the inner wall of a short length of duplex². Also double rings induced by low levels of protamine are incorporated into normal microtubules on polymerisation.

Further studies are in progress to test this model and to determine the relationship between the surface lattices of the various tubule forms.

Table 1 Diameters of the ring forms of tubulin

Type of ring	Conditions	Diameters (Å)*			
		Inner ring		Outer ring	
		Inner	Outer	Inner	Outer
Double ring	Tubulin + protamine at 4 °C	120	250	250	380
Double ring	Tubules + protamine cooled to 4 °C	120	250	250	380
Single rings	Tubules + protamine cooled to 4 °C	—	—	250	380
Single rings	Tubulin 4 °C	—	—	250	380

* Range of diameter measurements $\pm 20\%$.

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An outer component of microtubules

I HAVE described (ref. 1 and O.B., unpublished) a method by which microtubules from blood platelets or brain may be isolated in an intact form. They are preserved in a medium containing glycerol and dimethyl sulphoxide and can be collected in large amounts by differential centrifugation. This procedure differs from the usual methods of isolation which begin with unpolymerised tubulin, the protein of which microtubules are comprised^{2,3}, and has the advantage that structures associated with them in the cell are retained (O.B., unpublished). In the electron microscope these appear as a coating around the microtubule wall which is usually of an ill-defined amorphous nature (Fig. 1b).

Occasionally the microtubules show a distinctive structure of regular morphology (Fig. 1a). This is seen in negatively-stained preparations as a thickening of the microtubule wall, of limited extent longitudinally, in which the outer diameter is increased to 360-390 Å, but the inner lumen remains at 120-130 Å (Fig. 1a). The outer component often appears as stacked disks with a periodicity of 50 Å and inclined at an angle of 75° to the long axis, but sometimes a looser structure seems to be wound around the microtubule.

I found that a very similar kind of outer component could be induced at will. Treatment with various compounds caused the microtubules to aggregate and on examination in the electron microscope most of them were seen to have thickened walls. The compounds which did this were Alcian blue 8GX (1%), protamine sulphate (0.1 mg ml⁻¹), polylysines (molecular weights 1,000-30,000; 0.1 mg ml⁻¹) and DEAE-dextran (0.4 mg ml⁻¹), (ref. 4), all of which carry multiple positive charges at the pH of the stabilising medium. The outer components they produced were closely similar to those described above and were made of distinct globular subunits about 50 Å in diameter (Fig. 1c). In specimens fixed in glutaraldehyde and tannic acid⁵ and sectioned, the two layers of the wall were clearly visible (Fig. 1d).

Thus some isolated brain microtubules are decorated with a "natural" outer component but decoration can be induced on virtually all microtubules in a preparation by incubation with certain cationic compounds. There is no proof that the natural and the induced outer components are the same, but their ultrastructural appearance is very similar.

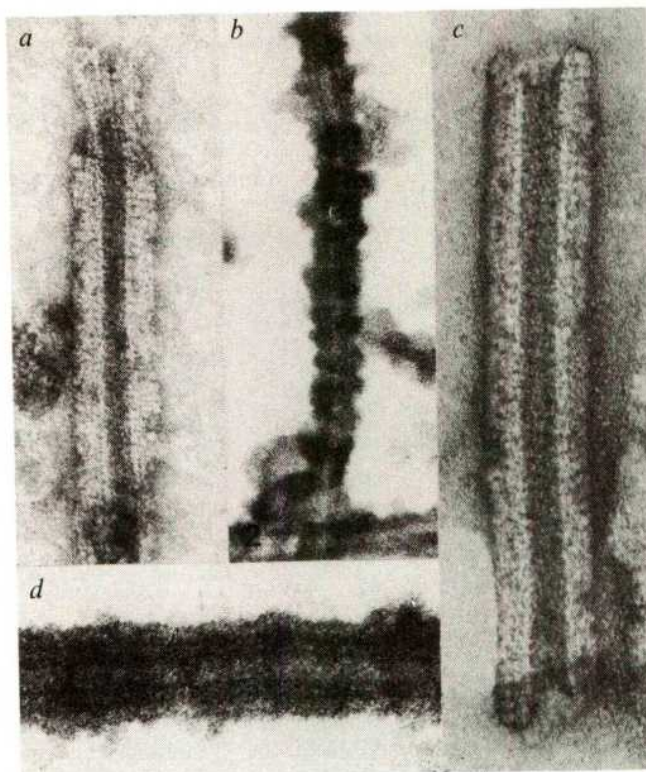


Fig. 1 a, Negatively-stained microtubule showing the outer component ($\times 250,000$). A crude preparation of brain microtubules was made in the following way. Cow brains were briefly homogenised in stabilising medium (50% glycerol, 10% dimethyl sulphoxide, 5 mM $MgCl_2$, and 5 mM phosphate buffer, pH 7), at room temperature. The homogenate was centrifuged, first at 12,000g for 20 min and then at 40,000g for 4 h. The final pellet was resuspended in a small volume of stabilising medium and samples were applied to carbon-coated grids. They were stained with 1% uranyl acetate and examined in the electron microscope. b, Longitudinal section of isolated microtubule ($\times 160,000$). The preparation was fixed in a mixture of 2% glutaraldehyde and 4% tannic acid⁵, without post-osmification, and embedded in araldite by standard techniques. c, Protamine-induced decoration of microtubule, negatively stained ($\times 300,000$). Crude preparations of brain microtubules prepared as in a (ref. 1 and O.B., unpublished), were resuspended in stabilising medium containing 0.1 mg ml⁻¹ protamine sulphate. They were incubated for 4-10 h in this medium before examination in the electron microscope. d, Longitudinal section of protamine-treated microtubule ($\times 240,000$). The preparation was fixed in a mixture of 2% glutaraldehyde, and 4% tannic acid as in b.

The outer component may also be formed on purified microtubules. Preparations which have been depolymerised, and polymerised in stabilising medium many times, and which are pure by SDS-gel electrophoresis, still give extensive decoration on treatment with cationic compounds. Similar observations have been made⁶ with microtubules that have been purified in a different way. Because the outer component seems to comprise a major part of the decorated tubules it seems reasonable to suppose that it can only be made of tubulin.

If the extra layer is composed of tubulin then it must be bound in a specific fashion. The subunits of the outer component are regularly placed and prolonged incubation with cationic compounds for up to 1 week does not result in the deposition of additional layers. Also, the sheets of tubulin which may be formed in some conditions⁶ become decorated only on one side, implying that only the outer side of the microtubule wall may be covered in this way.

The ease with which the outer component can be induced, and the fact that it is occasionally seen in freshly isolated microtubules, suggest that it could be present in the living cell. Structures of this precise nature have not been seen

in thin sections of cytoplasm but it may be relevant that microtubules often seem to be surrounded by a halo of low density⁷. This "clear space" from which other cytoplasmic constituents are excluded, has about the same width as the outer component, and it is not impossible that the two are in some way related.

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Foetal erythropoiesis in human leukaemia

DURING human development changes occur in the red-cell proteins and antigens¹. At birth the predominant haemoglobin (Hb) is Hb F with approximately 20% and less than 1% of the major and minor adult haemoglobin components (Hbs A and A₂) respectively. Hb F at birth is a mixture of two molecular species, one with glycine at position 136 of the γ chain ($\alpha_2\gamma_2^{136\text{Gly}}$) and the other with alanine at this position ($\alpha_2\gamma_2^{136\text{Ala}}$). The γ chains are designated γ and γ respectively and at birth the ratio of γ/γ is approximately 3:1 (ref. 2). Foetal red cells contain low levels of carbonic anhydrase isozymes B and C and react strongly with anti-i antibody but weakly with anti-I. During the first 12 months the level of Hb F falls and it is replaced by Hbs A and A₂. The ratio of γ/γ chains changes to 2:3 in the small amount of Hb F found in adult life³. The red cells lose their reactivity to i antibody and increase their reactivity to I. The carbonic anhydrase isozymes increase to adult levels by the end of the third year⁴. These changes act as markers for the change from foetal to adult erythropoiesis.

There are several examples of the emergence of foetal proteins during neoplastic transformation⁵. Since at least some human leukaemias involve red-cell as well as white-

cell precursors there may be a reversion to a foetal form of erythropoiesis in these disorders. The only condition where there is a marked increase in Hb F production is juvenile chronic myeloid leukaemia (JCML)⁶⁻¹⁰. We described⁷ a child with this disorder in whom there was a gradual increase in the Hb F level and a reduction in the amounts of Hbs A and A₂ and the carbonic anhydrase isozymes B and C over a period of 12 months. Thus as the disease progressed there was a gradual reversion to foetal erythropoiesis. In the study reported here we have confirmed this observation and shown that foetal erythropoiesis occurs occasionally in other forms of leukaemia.

Our subjects were nine infants or young children with JCML; 64 children and adults with acute myeloblastic leukaemia; 10 with erythroleukaemia; 19 with acute lymphoblastic leukaemia; 30 with chronic myeloid leukaemia and 44 with chronic lymphatic leukaemia. Haematological studies, haemoglobin and carbonic anhydrase isozyme analysis by starch gel electrophoresis, and Hb F and A₂ estimations followed previously described methods^{11,12}. The intracellular distribution of Hb F was assessed by acid elution¹³ or fluorescent anti-Hb F-antibody techniques¹⁴. The relative rates of α -, β - and γ -chain production were estimated by ³H-leucine incorporation¹⁵ and the glycine composition of peptides $\gamma 15$ or CB3 was determined as described previously^{2,16,18}.

The results of haemoglobin and red-cell enzyme analysis on the erythrocytes of 10 infants or children, nine with JCML and one with erythroleukaemia, are summarised in Table 1. The ages ranged from 3 months to 5 yr and the clinical and haematological findings were as in previously reported cases of JCML^{6,7}. The Philadelphia chromosome was absent in each case. In all but two cases high levels of Hb F in the 40-80% range were found, and these increased during the evolution of the illness. The Hb F was heterogeneously distributed among the red cells. In two cases where the fluorescent anti-Hb F-antibody technique was used the fluorescent cells constituted 60.3 and 100% of the total whereas the levels of alkali resistant haemoglobin were 30.2 and 69.5% respectively, indicating that a proportion of cells must have contained both Hbs A and F. In the cases with high levels of Hb F the glycine composition of peptides $\gamma 15$ or γCB3 ranged from 0.69 to 0.81; that is, in the range found in normal cord blood samples². In one case (case 8, Table 1) sequential estimations of the relative rates of α -, β - and γ -chain synthesis were obtained (Fig. 1). Initially γ chains made up approximately 45% of the total non- α -chains produced, which correlated well with the initial Hb F level of 38%. In a sample obtained 4 weeks

Table 1 Haematological data and haemoglobin and red cell enzyme analysis on 10 infants or young children with JCML or erythroleukaemia (case 8)

Case no.	Age (yr)	Sex	Days from presentation	Hb (g dl ⁻¹)	Platelets ($\times 10^6 \text{ l}^{-1}$)	WCC ($\times 10^6 \text{ l}^{-1}$)	Hb F (%)	Hb A ₂ (%)	Glycine residues in $\gamma 15$ or CB3	Carbonic anhydrase B and C
1	2	F	0	9.2	80,000	48,000	20.0	1.6	—	Reduced
			250	10.3	< 10,000	19,600	65.5	< 0.2	0.84	Absent
2	2	M	100	8.6	< 10,000	53,300	48.9	0.7	—	Absent
3	3 months	M	20	9.1	77,000	68,000	45.5	1.0	0.79	Reduced
4	5	M	10	10.2	15,000	15,700	50.0	< 1.0	0.81	Absent
5	3	M	5	8.2	8,000	42,000	38.2	0.98	0.69	Reduced
6	4	M	0	11.0	32,000	68,000	70.0	< 1.0	—	—
			60	10.6	36,000	7,200	69.9	Absent	0.83	Absent
7	5	M	0	11.0	38,000	15,700	30.2	< 0.02	0.75	Absent
			100	12.5	31,000	17,900	32.8*	< 0.2	—	Absent
8	3½ months	M	0	7.4	< 10,000	10,000	42.7	1.2	0.78	Absent
			28	7.9	< 10,000	1,800	85.0†	—	—	—
9	3 months	M	0	10.2	52,500	30,800	7.2	2.7	0.81	Normal
			450	9.6	20,000	36,800	1.9	3.1	—	—
			550	7.7	10,000	308,000	13.0	2.0	—	Normal
10	3 months	M	1	9.2	105,000	45,600	2.0	—	—	—
			100	11.0	200,000	35,000	4.9	1.4	0.56	Normal

* This child had 50% Hb F production by ³H-leucine incorporation studies at the time that the peripheral blood showed 32.8% Hb F.

† Obtained from ³H-leucine incorporation data.

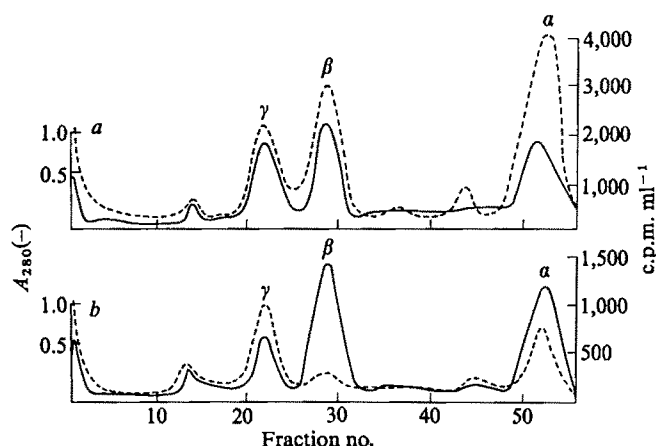


Fig. 1 Elution profiles of globin chains separated by CM-cellulose chromatography in a 8 M urea-mercaptoethanol-phosphate buffer system, pH 6.7. Peripheral reticulocytes and nucleated red cells were incubated for 1 h with ^3H -leucine in the conditions indicated in the text. Cells were then washed, lysed and total cell globin was prepared by the acid-acetone method. The globin chains were separated and the radioactivity incorporated into each chain was determined. Profile *a* is taken from a child with erythroleukaemia just after presentation. Profile *b* was prepared from the cells of the same child 28 d later. There is more β chain carrier in *b* because the child was receiving blood transfusions but it is clear there has been almost a total reversion to γ chain synthesis during the evolution of the illness.

later, γ chains constituted about 80% of non- α -chains produced, indicating that shortly before he succumbed he was producing more than 80% Hb F.

The highest levels of Hb F in the infants and children with JCML and erythroleukaemia were associated with a reduction in the levels of Hb A₂ and carbonic anhydrase B and C (Table 1) and in several cases none was detectable. In these cases the starch gel electrophoretic patterns of the red cell lysates were indistinguishable from those of normal newborn infants. Embryonic haemoglobin was not present. In these cases there was an elevation in the i antigen titre but only in one was there a significant fall in the level of I. Case 6 showed in addition to 70% Hb F, about 3% Hb Barts, but family studies indicated that in addition to having JCML he had received an α -thalassaemia gene from his mother. No haematological abnormalities were found in any other family members and the identical twin in case 3 was completely normal.

Cases 9 and 10 did not follow the pattern outlined above. Case 9 survived much longer, had a higher white cell count and his Hb F level rose to only 13%. Case 10, while having a clinical presentation identical in every way to JCML, ran a milder course and did not produce more than 4% Hb F. Peptide $\gamma 15$ prepared from the latter had a glycine composition of 0.56 residues which is in the range found in the small amounts of Hb F present in normal adults³.

Elevated levels of Hb F were observed also in 43 out of 64 patients with acute myeloblastic leukaemia, 7 out of 8 patients with erythroleukaemia, 12 out of 19 with acute lymphoblastic leukaemia, 19 out of 30 with chronic myeloid leukaemia and 15 out of 44 with chronic lymphatic leukaemia. In most cases the level did not exceed 5% of the total haemoglobin and only in three cases were values in the 10–25% range encountered. No consistent alterations in the levels of Hb A₂, carbonic anhydrase isozymes or I antigens were found. The glycine composition of $\gamma 15$ of the Hb F varied widely, as has been noted by others¹⁰. Hb F levels were followed in a group of 17 patients with acute myeloblastic leukaemia for periods ranging from 1 to 15 months. Fifteen of the seventeen developed an increase in the level of Hb F about 60 d after the commencement of treatment at a time when, following bone marrow aplasia due to

chemotherapy, rapid regeneration was taking place. Significantly greater increases of Hb F levels were observed in those achieving a remission. Details of these patients will be reported elsewhere.

The results indicate that in some forms of leukaemia in early childhood there may be an almost total reversion to a foetal form of erythropoiesis. Levels of Hb F may reach those of cord blood. The levels of Hb A₂ and carbonic anhydrases B and C fall to produce an electrophoretic pattern of red-cell proteins identical to that observed in newborns. This pattern of erythropoiesis, found most commonly in JCML, can occur also in erythroleukaemia and it does not occur in every case of JCML. This may, of course, simply reflect the heterogeneity of JCML²⁰. It was not seen in other forms of leukaemia and the slight elevation of Hb F in these disorders is associated in many cases with recovery from bone marrow aplasia caused by chemotherapy. When the reversion to foetal erythropoiesis occurs it provides a marker for the presence of the neoplastic cell line.

Why does erythropoiesis revert to a foetal form during the evolution of some types of leukaemia in early childhood? It is possible that it is another example of the reversion to foetal protein synthesis which occurs with other neoplasms⁵. If this is the case, however, there is no reason why it should occur much more frequently in infancy. Its occurrence at this time suggests that it is related to the normal mechanism of differentiation of red-cell proteins during maturation. Although by the end of the first year most erythrocytes contain mainly Hb A, cell lines persist which produce relatively large quantities of Hb F^{14,21}. Such 'F cells' are produced in adults in relatively constant numbers, suggesting that some stem-cell lines do not undergo the normal switchover from Hb F to Hb A production. It has been suggested that this switch is hormonally controlled^{1,22} and that the 'F cell' population in adults has arisen by chance in stem cells which did not come under its influence. Thus the chances of a leukaemic transformation occurring in a line making predominantly Hb F would be much greater in infancy and therefore the reversion to foetal erythropoiesis should be much commoner at this age. Similarly the chances of it occurring in adults would be extremely small although an occasional case might occur²³. Thus the findings are fully consistent with the idea⁷ that these leukaemias arise in cell lines which have escaped the normal processes of differentiation during development.

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Evidence for Hb Lepore-like hybrid globin β chain genes in mice

We have described structural data for mouse and rabbit embryonic β -like ϵ chains and the conclusions which can be drawn with respect to the evolution of ϵ globin chain genes in both species¹. Here we present evidence for crossover events in the ancestral mouse β and ϵ -type chain genes leading to Hb Lepore-like globin chain genes which are functional in mice of recent evolutionary origin.

BALB/c mice, which we used for this study, have two types of embryonic β -like ϵ chains— $\epsilon\gamma$ and $\epsilon\zeta$ (refs 2–7). Based on the composition of tryptic peptides of the $\epsilon\gamma$ and $\epsilon\zeta$ chains,

the tentative sequence of both chains was established using the adult mouse β chains as a reference^{2,5,8,9}. BALB/c mice have two linked genes for the adult β chains, which control the synthesis of the β major and minor globin chains, respectively. The two chains differ at six positions (Nos 9, 16, 20, 58, 72 and 76)⁸.

$\epsilon\gamma$ and β minor chains are closely related since they are identical at four of the six positions at which β major and minor are different. The other two positions are different in $\epsilon\gamma$ from both β major and β minor^{1,2}. Therefore the β minor chain was used for comparison of the structure of the ϵ and β chains. $\epsilon\zeta$ and $\epsilon\gamma$ chains are unrelated apart from their β -like character¹. A comparison of the $\epsilon\gamma$ and β minor chains with respect to the sites of amino acid substitutions shows that most substitutions are located at the amino and also some at the carboxy-terminal ends of the $\epsilon\gamma$ chain, whereas the central part of the chain is almost identical to the β minor chain (Fig. 1).

Rather the reverse is true for the $\epsilon\zeta$ and β minor chains. Here the substitutions are concentrated at the centre of the $\epsilon\zeta$ chain whereas both the amino and carboxy terminal ends look very similar to the β minor chain. Since some peptide composition data of both ends of the ζ chain are, however, still missing, the situation is not as clear as for the $\epsilon\gamma$ chain. The presence of an amino terminal peptide though, which is identical to the adult β -chain peptide composition, leads us to predict close resemblance of the amino terminal part of the $\epsilon\zeta$ chain and of the β minor chain (Fig. 1).

The reciprocal nature of the two ϵ chains as compared with the adult β chains as shown in Fig. 1, suggests that either the β -chain genes or the ϵ -chain genes arose by a crossover event between non-identical genes. Either a single or a double crossover could explain our data. The double crossover interpreta-

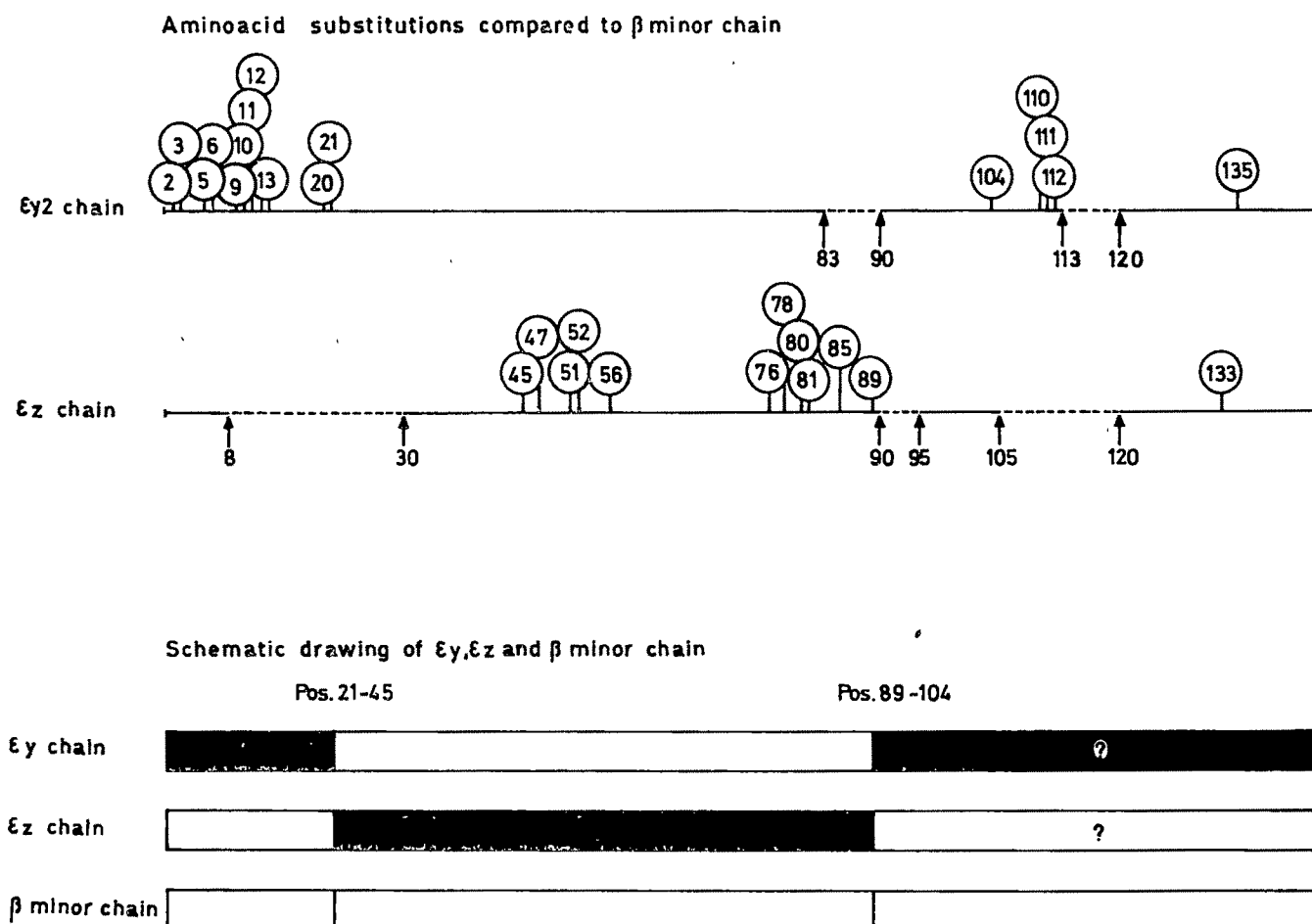


Fig. 1 Unequal distribution of amino acid substitutions is found in the embryonic $\epsilon\gamma$ and $\epsilon\zeta$ chains of the mouse as compared with the adult β minor chain. This suggests an origin of either adult β -type or embryonic ϵ type chains by crossover mechanisms, as

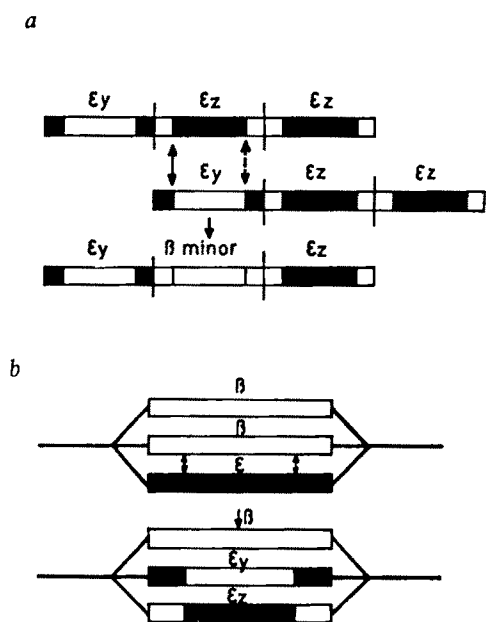


Fig. 2 Crossover models which may account for the presence of Hb Lepore-like hybrid globin β or ϵ -chain genes in mice. Two theoretical possibilities can be assumed for the distribution of amino acid substitutions as shown in Fig. 1. (1) Two ancestral genes, that is the ancestral ϵy and ϵz -chain gene form one new gene, the β minor chain gene. (2) Two ancestral genes that is the ancestral ϵ and β -chain gene undergo a crossover event thus creating two new genes, the ϵy and ϵz -chain genes. In principle two models can account for the observed phenomenon of Hb Lepore-like hybrid globin chains. First, the model of misalignment and unequal crossover of closely related genes, which is normally used for the explanation of Lepore haemoglobins. Second, a crossover mechanism involving identical and non-identical genes arranged in parallel on one chromosome. A model of parallel arranged DNA double strands located within one chromosome has for example been proposed by Smithies for the genes of immunoglobulin chains¹¹. The first crossover model of misalignment and unequal crossover only applies to hypothesis (1), that is, two ancestral genes form one new gene at least under the premise of one temporal event. Otherwise one has to assume additional processes of genetic segregation and recombination first separating and then bringing together again the two newly formed genes. The second crossover model involving genes arranged in parallel would account for hypothesis (2), namely two ancestral genes create two new genes, especially under the additional assumption of one single temporal step.

tion would have to be supported by more structural data, mainly on the ϵz chain.

Two main mechanisms are considered here to interpret the crossover origin of mouse β or ϵ -chain genes. The first mechanism would involve unequal Lepore-type crossing over between related non-allelic genes¹⁰, the second would involve reciprocal crossing over between allelic non-identical but closely related genes arranged in parallel on the same chromosome¹¹ (Fig. 2).

We made use of minimal assumptions for explanation of the data. A single temporal event using the generally accepted colinearity of all genes would be the easiest interpretation. If one accepts one single meiotic crossover event to account for the structure of all three β -like genes, ϵy , ϵz and β minor, then the most likely origin would be explained by the Lepore hypothesis (Fig. 2). We assume that the ϵy -chain gene is the ancestral ϵ -chain gene, since ϵy is the only ϵ chain which combines with the embryonic α -type chain χ to form haemoglobin E_1 . The ϵz chain apparently only combines with the adult α chain^{2,3}. Then the ϵz chain might have been the ancestral adult β -type gene. An unequal crossing over (Fig. 2) between these two genes could have generated the adult β gene (anti-Lepore) which was closer to β minor than to β major. A further independent duplication of β minor would then have been the basis for the evolution of an independent β major gene.

This Lepore hypothesis has to be modified if a double crossover would have to be assumed to account for the reciprocal nature of three pieces of the ϵy and ϵz -chains as compared with the β chain (Fig. 1). A prerequisite for this model would be that the ϵz -chain gene was duplicated before the double crossover event. After the double crossover, the β -chain gene would then be between the two ϵ -type genes. This would naturally have consequences for the regulation of coordinated expression of both ϵ -chain genes.

The hypothesis of parallel not colinear genes, if extended to the β -globin loci, would not present any difficulties in the creation of the ϵy , ϵz and β -chain genes, either in meiotic or in other germ line cells. No difficulty would arise for single or double crossovers. No loss or gain of genetic material would occur (Fig. 2). In this model, the β -chain genes do not need to be the result of a crossover between ϵy and ϵz -chain genes. A reciprocal crossover between an ancestral β and ϵ -chain gene could have formed the ϵy and ϵz -chain genes (Fig. 2). The hypothesis just described and the hypothesis of unequal crossover for the origin of Lepore-type globin chains can be checked in patients with Lepore haemoglobin, especially in the homozygous state. The data for one homozygous individual with Hb Lepore Boston¹⁰, who shows no detectable amounts of anti-Lepore globin seem to favour the unequal crossover model.

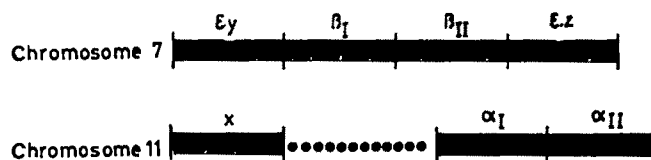


Fig. 3 Hypothetical arrangement of α and β -type chain genes of BALB/cJ mice on chromosome 11 and 7, respectively. The location of adult α and β -chain genes on their respective chromosomes is known, as is the tight coupling of ϵy and adult β -chain genes. From our crossover hypothesis involving ϵy , ϵz and adult β -chain genes, a coupling of all β and ϵ -chain genes seems to be likely as well as the location of adult β -chain genes between embryonic β chain genes. The information on location and coupling of α -type chain genes was included for the sake of completeness¹⁵. The assumption that the χ chain gene is linked to the adult α globin chain genes is based on structural homology to the adult α chains and also on functional aspects such as mutual replacement of χ and α chains during ontogenesis¹⁶.

Lepore-type β chain has been reported in a species of mouse (*Mus musculus caroli*)¹². Therefore it seems possible that crossover events have been important in the evolution of murine β -chain genes.

In rabbits as in mice, evidence has been obtained for multiple embryonic β -type ϵ chains¹. These chains look much more similar to each other and the distribution of amino acid substitutions compared with the adult β chains of rabbits seems more even than in mice. Therefore we assume that no crossover was involved in the generation of the β -type genes in the rabbit.

The likelihood that either the existing β -chain genes of BALB/c mice or possibly both ϵ -chain genes arose by recombination events suggests that both ϵ -chain genes are closely linked with the β -chain loci (Fig. 3). Linkage of the β minor and β major chain genes^{12,13} and of both to ϵy ^{4,7} on chromosome 7¹⁴ has been shown before.

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Embryonic ϵ chains of mice and rabbits

THE existence of an embryonic α -type globin chain (χ or ζ) in early embryos of mice and rabbits has been described¹⁻³. A similar ζ chain can be found in humans^{4,5}. Here we present data on the structure of the embryonic β -type ϵ chains of mice and rabbits. In mice, three embryonic haemoglobins, Hb EI, EII and EIII have been described. These haemoglobins consist of four globin chains, namely the embryonic χ , ϵ and ϵ chains and the adult α chain^{3,6-8}. Hb EI has the structure $\chi_2 \epsilon_2$, Hb EII is $\alpha_2 \epsilon_2$ and Hb EIII is $\alpha_2 \epsilon_2$. The χ chain is an embryonic α -type chain^{1,2}. We find six haemoglobin fractions in nucleated embryonic erythrocytes of the rabbit. Three of the haemoglobins (Hb EI-EIII) do not contain α , but χ chains like Hb EI of the mouse; the other three (Hb LI-III) are similar to mouse Hb EII and EIII¹.

All non- α -type embryonic globin chains of both species are β -type ϵ chains. We find less structural similarity between the ϵ chains of different species than between the β and ϵ chains of the same species. This indicates, in contrast to the embryonic χ chains, that ϵ chains diverged by independent evolution from the ancestral β chains of mice and rabbits.

The ϵ chains of 12-13-d-old embryos of BALB/c mice and of 14-d-old embryonic New Zealand white rabbits were digested by trypsin, and the tryptic peptides were separated by the fingerprinting method. The amino acid composition of the tryptic peptides is shown in Table 1. They were compared with the β chain sequences of mouse (β major and β minor)^{9,10}

and rabbit¹¹⁻¹³. According to the principle of least dissimilarity, the best correspondence was obtained using the β chains of each species as a reference (Fig. 1). The ϵ chain which has erroneously been called ϵ chain previously¹, shows a closer overall homology to the β minor than to the β major chain. In addition, in 4 out of 6 positions, that is in positions 16, 58, 72 and 76, in which the β major and minor chains are different, the ϵ and β minor chains are identical. The other two positions are different from both β major and minor chains. For the ϵ chain, similar conclusions concerning the relationship to either of the two adult β chains are not yet possible because of a lack of data.

In the case of the rabbit, different chromatographic fractions of ϵ chain material have been analysed separately by the fingerprint method. The fractions were, however, not resolved sufficiently for conclusions to be drawn as to how many rabbit ϵ chains are present. All the data were therefore pooled (Table 1, Fig. 1). These peptide compositions were compared with the adult globin chains of rabbits. The closest homology was found using the rabbit β chain as a reference as shown in Fig. 1. The homologised sequence shows close similarity to the adult β chain. At several positions, indicated by brackets, two or three different amino acids had to be placed either because peptides with very similar compositions were found at different spots of the fingerprint or because one peptide showed non-integral values for two different amino acids. This proves the presence of more than one rabbit ϵ chain. The degree of homology of rabbit ϵ and rabbit β is similar to mouse ϵ and β chains.

To get an impression whether a common evolutionary origin of rabbit and mouse chains could be supported by our data, we compared the individual peptide compositions of mouse and rabbit β chains⁹⁻¹³ with the corresponding peptides of the ϵ chains (Table 1, Fig. 1). For comparison, we used the mouse β minor chain as reference chain because of close structural relationship to one murine embryonic ϵ chain, namely the ϵ chain. In the case of the rabbit, the β chain sequence, as reported by Braunitzer *et al.*¹¹ was taken as reference because it looked somewhat closer to the embryonic ϵ chains of rabbits (positions 52 and 56, Fig. 1) than the allelic sequence^{12,13}. Since we used peptide compositions for comparison, even in the case of established sequences, minimal

Amino acid/ Pos no	1	24	73	96	
β rabbit	Val His Leu Ser Ser Glu Glu Lys/Ser Ala Val Thr Ala Leu Trp Gly Lys/Val Asn Val Glu Glu Val Gly		Glu Gly Leu ^{Asn} Ser His Leu Asp Asn Leu Lys/Gly Thr Phe Ala Lys/Leu Ser Glu Leu His Cys Asp Lys/Leu		
ϵ rabbit	(Gly Ser) Met Asx ^[Leu] [Ile] Pro Asx ^[Ser] [Ala] Phe Asx Gly ^[Thr] [Ile]		Gly Phe Lys/Lys ^[Val] [Met] Ala		
χ mouse	His Leu Asx Ala		His Asx Ser Glu Ala Val Phe		
γ mouse	Asx Phe Ala Glu Thr Leu Ile Asx Gly Ala Val Glu		Lys/? Leu Asx Leu		
β mouse	Val His Leu Thr Asp Ala Glu Lys/Ser Ala Val Ser Cys Leu Trp Ala Lys/Val Asn ^{Pro} Ser Asp Glu Val Gly		Ser Gly Leu ^{Lys} His Asn Leu Asp Asn Leu Lys/Gly Thr Phe Ala Ser Leu Ser Glu Leu His Cys Asp Lys/Leu		
Amino acid/ Pos no	25	48	97	120	
β rabbit	Gly Glu Ala Leu Gly Arg/Leu Leu Val Val Tyr Pro Trp Thr Gln Arg/Phe Thr Glu Ser Phe Gly Asp Leu		His Val Asp Pro Glu Asn Phe Arg/Leu Leu Gly Asn Val Leu Val ^{Val} Ile Val Leu Ser His His Phe Gly Lys/		
ϵ rabbit		Thr Pro? Ala Lys/ ^[Gly Ala Phe] ^[Glu Ser Ile] ^[Asx Val] ^[Glu Leu]			
χ mouse		Leu Glu			
γ mouse		Phe Asx			
β mouse	Gly Glu Ala Leu Gly Arg/Leu Leu Val Val Tyr Pro Trp Thr Gln Arg/Tyr Phe Asp Ser Phe Gly Asp Leu		His Val Asp Pro Glu Asn Phe Arg/Leu Leu Gly Asn Met Ile Val Ile Val Leu Gly His His Leu Gly Lys/		
Amino acid/ Pos no	49	72	121	144	146
β rabbit	Ser Ser Ala ^{His} Asn Ala Val Met ^{Ser} Asn Pro Lys/Val Lys/Ala His Gly Lys/Lys/Val Leu Ala Ala Phe Ser		Glu Phe Thr Pro Gln Val Gln Ala Ala Tyr Gln Lys/Val Val Ala Gly Val Ala Asn Ala Leu Ala His Lys/Tyr His		
ϵ rabbit	^[Ile] ^[Ser] ^[Asx Ala] ^[Asx Thr] ^[Lys/Gly] ^[Met Asx] Tyr		^[Glu] ^[Asx] ^[Ser] ^[Ala] ^[Ser] ^[Leu] ^[Ile] ^[Ala] ^[Val] ^[Thr] ^[Ile]		
χ mouse	Leu Thr Val Ala		Glu Glu Ala Ala		
γ mouse	Ala Ser Gly Pro		Glu Val Ser		
β mouse	Ser Ser Ala Ser Ala Ile Met Gly Asn ^{Pro} Lys/Val Lys/Ala His Gly Lys/Lys/Val Ile Thr Ala Phe ^{Glu} Asn		Asp Phe Thr Pro Ala Ala Glu Ala Ala Phe Gln Lys/Val Val Ala Gly Val Ala Thr Ala Leu Ala His Lys/Tyr His		

Fig. 1 Sequence homology of mouse and rabbit ϵ chains as compared with the corresponding adult β chains. The ϵ chain peptide in position 91-95 was taken from Gilman¹⁴. Gilman's sequence data in addition to our peptide data were used for positions 1-30 of the ϵ chain¹⁴. In the case of the rabbit, two or three different amino acids had to be placed at the same positions as indicated by brackets. This proves the presence of more than one rabbit ϵ chain. Serine and glycine have been found in addition in the rabbit ϵ chain peptide homologised to the adult β T1 peptide. It seems possible that both residues are linked to the N terminal end of the rabbit ϵ chain(s), as has been found for a minor fraction of adult rabbit α chain, for the embryonic rabbit α chain²⁰ and for the embryonic rabbit α chain²¹.

differences were determined for all chains. For the calculation we omitted those peptides for which the homology was doubtful. We therefore used for comparison the following homologised stretches: positions 41–82, 96–104 and 121–146 (Fig. 1). In the case of the rabbit for positions 41–59, only the corresponding well fitting peptide was included in the comparison

(Table 1). The $\epsilon\gamma_2$ chain of BALB/c mice has probably asparagine or some unknown residue at position 77, whereas the $\epsilon\gamma_1$ chain may have either lysine or arginine¹⁴. From our data, the presence of asparagine can be excluded (Fig. 1). Of a stretch of peptides totalling 77 amino acids we find the closest homology between mouse β and $\epsilon\gamma$ (3/77 difference) β and $\epsilon\gamma$

Table 1 Amino acid composition of the tryptic peptides of mouse and rabbit ϵ chains

a Rabbit - Residue no which homologised to known β chain sequences

Amino acid	1-8		9-17		18-30		31-55		56-59		41-59		60-61		62-65		66-77		67-77		78-82		78-82		83-87		88-95		96-134		105-120		121-132		121-132		121-132		133-144		133-144		145-146										
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b											
Lysine	09	1	10	1			20	2	10	1	11	1	11	1	10	1	10	1	19	2	09	1	08	1	10	1	12	1	10	1	22	2	19	2	04	0	08	1	0 ^a	0	10	1	10	1									
Histidine																10	1																																				
Arginine					07	1																																															
Aspartic Acid	16	2	10	1	15	1	20	2	11	1	29	3									23	2	23	2			22	1	18	2	19	2	04	0	08	1	0 ^a	0	10	1	10	1											
Threonine			10	1	06	05	20	2			05	1																10	1	13	1	10	1	11	1	11	1	10	1	10	1												
Serine	28	3	05	05			10	1			35	3																10	1			12	1	05	0	10	1	20	2														
Glutamic Acid					31	3					20	2																10	1	10	1	10	1	40	4	(3)	3	28	3														
Proline	+	1					?	1	+	1	11	1																				+	1			+	1	08	1														
Half Cystine																												*	1					*	1																		
Glycine	10	1	10	1	37	4	23	2	09	1	13	1			10	1				22	2					07	1					09	1							10	1	09	1										
Alanine			15	15	10	1	39	4			12	1			08	1				20	2					23	2					19	2	31	3	(2)	2	(3)	2	43	4	33	3 ^a										
Valine			11	1	08	1	42	4			06	1	09	1						10	1	06	1																														
Methionine	03	1									05	1																																									
Isoleucine	05	05	05	0	08	05	11	1			06	1																																									
Leucine	05	05	09	1	09	1	19	2			08	1								09	1	09	1	10	1																												
Tyrosine																				09	1																																
Phenylalanine			10	1			27	3			16	2								15	2																																
Tryptophane																																																					
No. of residues	10	9	13	25	4	19	2	4	1	11	5	5	5	8	9	16	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	2					
yield (10 ⁻⁹ M)	8	15	12	45	6	23	68	85	265	18	18	44	48	6	30	6	13	17	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	2				

b Mouse ϵ -chain Residue no which homologised to known β chain sequences

Amino acid	1-8		18-30		31-40		41-59		60-61		62-65		66		67-76		78-82		96-104		105-112		121	132	133	144	145	146
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Lysine	11	1					09	1	10	1	10	1	10	1	11	1	10	1	08	1	09	1	10	1	10	1		
Histidine											09	1							07	1					11	1	+	1
Arginine			10	1	08	1																						
Aspartic Acid	11	1	10	1			27	3									23	2	18	2			06	1	10	1		
Threonine	10	1			10	1									12	1					08	1	13	1	09	1		
Serine							37	4							09	1					07	1			07	1		
Glutamic Acid	19	2	33	3	08	1									09	1			07	1			21	2				
Proline					+	1	+	1											+	1			+	1				
Half Cystine																												
Glycine			29	3			19	2			08	1			11	1					08	1			09	1		
Alanine	10	1	08	1			18	2			07	1			09	1							47	4	33	3		
Valine	10	1	30	3	17	2			10	1					09	1			09	1					27	3		
Methionine							06	1													07	1						
Isoleucine							07	1							09	1												
Leucine			09	1	18	2	10	1							08	1	18	2	08	1	15	2			15	1		
Tyrosine					+	1	+	1																			+	1
Phenylalanine	09	1					2								10	1			+	1			17	2				
Tryptophane					*	1																						
No. of residues	8	13	10	19	2	4	1	10	5	9	8	12	12	2														
yield (10 ⁻⁹ M)	5	4	2	2	10	2	4	3	10	2	3	10	2	3	10	2	3	10	2	3	10	2	3	10	2	3	10	2

c Mouse ϵ -chain Residue no which homologised to known β chain sequences

Amino acid	1-8	31-40	41-59	60-61	62-65	66	67-82	83-89	96-104	121-132	133-144	145-146
Lysine	09	1	10	1	14	1	09	1	09	1	10	1
Histidine	07	1					11	1				
Arginine												
Aspartic Acid	14	1		21	2							
Threonine	07	1	14	1	10	1						
Serine				32	3							
Glutamic Acid	10	1	14	1	04	1						
Proline			+	1								
Half Cystine												
Glycine				04	1		11	1				
Alanine	08	1		16	2		07	1				
Valine	06	1	15	2	06	1	11	1				
Methionine				09	1							
Isoleucine				12	1							
Leucine	12	1	21	2	33	3						
Tyrosine		05	1	+	1							
Phenylalanine			+	1								
Tryptophane												
No. of residues	8	10	19	2	4	1	16	7	9	12	12	2
yield (10 ⁻⁹ M)	10	7	7	12	20	10	8	6	8	10	8	8

The embryonic ϵ globin chains of mouse and rabbit were separated^{1,2}. The embryonic ϵ chains were digested with trypsin and after separation of the tryptic peptides by fingerprinting, the amino acid composition analysis was carried out^{1,2}. Line 1 indicates the residue numbers that were homologised to known β chain sequences. No peptide characteristic of adult β chains have been found.

a, Residues; b, Nearest whole number of residues

|| Value not certain, short column was switched off later than usual.

* Prolin is present

** Not present, destroyed during hydrolysis

†† Value for Asx too high, probably because of contamination.

‡‡ Peptide no. 133-144B was probably contaminated by peptide 133-144A.

© Presumably N terminal, partly destroyed.

Values in brackets are estimates because of difficulties with the chromatographic separation or integration.

(10/77) and $\epsilon\gamma$ and $\epsilon\zeta$ (12/77). Almost equally distant seem to be rabbit β and rabbit ϵ (13–14/77) and rabbit ϵ as compared with mouse $\epsilon\gamma$ (14–16/77) and $\epsilon\zeta$ (16–18/77).

In summary, our structural data on embryonic globin chains of mammals show the following characteristic features: in all vertebrate species which have been studied^{2–4,5,7}, there are very early embryonic haemoglobins which contain neither α nor β , but β -like ϵ and α -like χ chains. The χ chain is completely replaced during later yolk sac embryogenesis by the adult α chain. The structure of the χ chain of different species (mouse, rabbit^{1,2}, man^{4,5}) seems to be closely related. No obvious homology apart from their β -like character could be detected for the embryonic ϵ chains of mice and rabbits. Surprisingly, $\epsilon\zeta$ and $\epsilon\gamma$ seem to be more different from each other than from the adult mouse β chain. This might be a consequence of a recent crossover event leading to the β and ϵ chain genes in mice¹⁵. The scarce data on the human ϵ chain¹⁶ do not permit a definitive answer. The human data (ref. 17 and A. Yoshida, personal communication) seem, however, to confirm our impression that mammalian ϵ chains are not very far removed from β chains in contrast to χ and α chains. This failure to find common similarities of the different chains suggests that ϵ or β chains have evolved separately in closely related species such as rabbits and mice, or that ϵ and β chain continuously coevolve¹⁵.

The evolutionary stability of embryonic χ chains and the probably independent evolution of different ϵ chains as well as their close similarity to the β chains suggests that the distinctive functional features of embryonic haemoglobins are due mainly to the χ chains¹⁸. The χ chains may therefore contribute essentially to the high oxygen affinity of embryonic mouse haemoglobins¹⁹.

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Predicted distribution of NAD domain among glycolytic enzymes

X-RAY crystallographic studies have indicated that four dehydrogenases^{1–4}, including lactate¹ and glyceraldehyde phosphate⁴ dehydrogenase, contain a central parallel β sheet consisting of six strands flanked by four α helices. This supersecondary structure⁵ is called the dinucleotide fold or nicotina-

mid adenine dinucleotide (NAD) domain since it forms an NAD-binding site at the C terminus of the β sheet. The dinucleotide fold can be considered as two roughly identical mononucleotide domains each containing about 60 residues arranged in an alternating $\beta\alpha\beta\alpha$ sequence and related by an approximate twofold axis⁵. The aromatic specificity site of subtilisin^{6,7} and the flavin-binding site of flavodoxin^{8,9} seem⁵ to be formed by a secondary structure very similar to a mononucleotide domain of the dehydrogenases. Crystallographic studies have shown that the structures of phosphoglycerate kinase^{10,11}, hexokinase¹², adenylate kinase¹³, phosphoglycerate mutase¹⁴ and triosephosphate isomerase¹⁵ also contain β sheets containing at least five strands flanked by at least three α helices. The nucleoside phosphate or, in the case of the mutase and isomerase, the sugar phosphate-binding site for each of these enzymes is either known^{10–12}, or strongly suspected^{13–15} to be located at the C terminus of the β sheet. Differences occur, however, in some of these supersecondary structures relative to the NAD domain of the dehydrogenase family as regards the direction of the β strands¹², the location of the binding site relative to the plane of the β sheet¹², and most significantly, the sequential order or connectivity of the individual strands in the β sheet^{12–14}. Whether these related supersecondary structures represent convergent^{5,12} or divergent evolutionary processes^{4,16}, or both¹⁷, is a topic of active debate.

We have demonstrated¹⁸ that blue dextran–Sephacryl chromatographic columns seem to function as affinity columns specific for proteins whose nucleoside phosphate-binding sites are constructed by the NAD domain or by a supersecondary structure having a connectivity similar to that of the NAD domain and whose apolar pocket is located above the β sheet. If so, such affinity chromatography can be used to predict the presence of such supersecondary structures in proteins whose high resolution crystallographic structures have not been

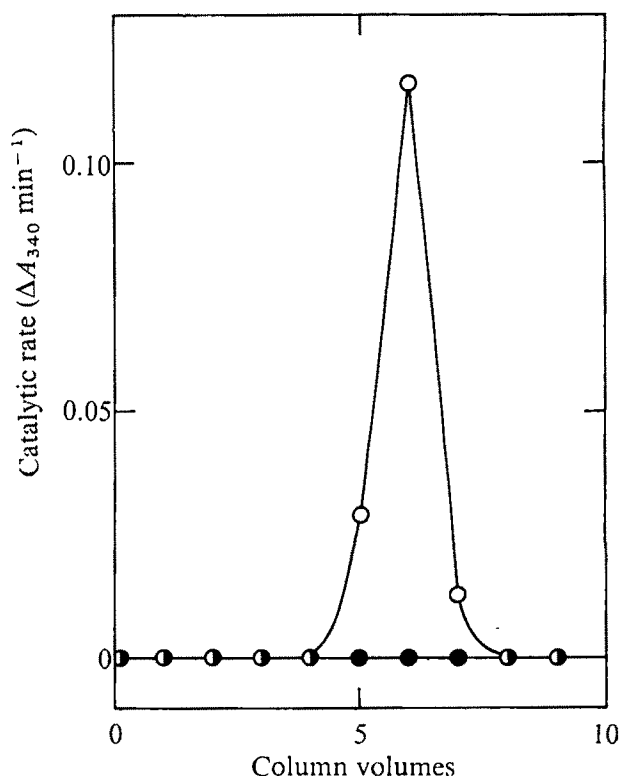


Fig. 1 Elution profile of aldolase from a blue dextran–Sephacryl affinity column. About 100 μ l solution of aldolase containing about 100 μ g protein in 10 mM Tris-HCl buffer, pH 7.5, was applied at zero effluent volume to a 1 ml affinity column equilibrated with the same buffer. The column was then washed with five 1 ml aliquots of the buffer and with five aliquots containing either 10 mM fructose-1,6-diphosphate (○), or 100 mM NaCl (●). Enzymic activity of aliquots of effluent fractions was measured using the standard coupled spectrophotometric assay procedure.

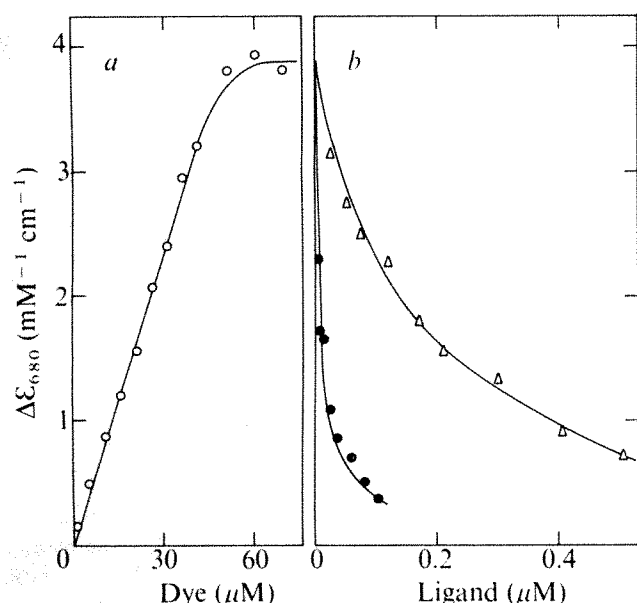


Fig. 2 Spectral measurements of aldolase-dye complexes. *a*, Difference spectral titration of aldolase with dye. Identical aliquots of a concentrated solution of Cibacron blue F3GA dye were added to the same volumes of a 23 μ M aldolase subunit solution in 10 mM Tris-HCl buffer, pH 7.5, and to the solvent. *b*, Difference spectral titration of aldolase-dye complex with ligands. Increasing concentrations of fructose-1,6-diphosphate (●) or NaCl (△) were added stepwise to a 23 μ M aldolase subunit solution in 10 mM Tris-HCl buffer, pH 7.5, containing 77 μ M dye and to a 77- μ M dye solution in the same solvent. Extinction values are in terms of protein subunit concentration corrected for dilution.

reported. Since crystallographic studies have clearly demonstrated that several glycolytic enzymes contain the NAD domain, we have utilised affinity chromatography to predict the distribution of the NAD domain or close analogues thereof among all the enzymes in the glycolytic pathway.

Results of such a chromatographic survey, summarised in Table 1, indicate that phosphorylase *a*, phosphofructokinase, fructose diphosphatase, aldolase and pyruvate kinase specifically bind to the blue dextran-Sepharose chromatographic columns at pH 7.5 and is eluted by a concentration of its specific phosphorylated substrate or effector at least an order of magnitude less than the concentration of the nonspecific ligand, NaCl. A typical elution profile is illustrated in Fig. 1. Although enolase also binds to blue dextran columns at pH 7.5, it is eluted equally well by solutions containing the same ionic strength of specific ligands, phosphoenolpyruvate or 2-phos-

phoglycerate, or the nonspecific ligand, NaCl. Such behaviour is characteristic of ion-exchange chromatography and is not indicative of a specific interaction. By contrast, hexokinase, either in the presence or absence of 10 mM glucose, phosphoglucose mutase, triosephosphate isomerase, and phosphoglucose isomerase do not bind to the chromatographic columns at pH 7.5.

Conclusions as to the formation of specific enzyme-immobilised dye complexes are supported by difference spectral measurements using the free dye of blue dextran, Cibacron blue F3GA. Complexing of the free dye with lactate dehydrogenase and phosphoglycerate kinase produces difference spectra having positive maxima in the range 660–680 nm, presumably due to insertion of the dye into the hydrophobic pockets of the NAD site formed by the NAD domain¹⁹. The hyperbolic dependence of the difference maximum extinction at 680 nm resulting from addition of the free dye to aldolase (Fig. 2) indicates the formation of a discrete enzyme-dye complex. Since the substrate, fructose-1,6-diphosphate, is much more effective than the nonspecific ligand, NaCl, in diminishing the difference extinction (Fig. 2), it is likely that the free dye specifically complexes with aldolase at its substrate sites. By contrast, the nonspecific ligand, NaCl, is equally effective on an ionic strength basis with the ligands ATP and 2-phosphoglycerate in diminishing the difference extinctions of hexokinase-dye and enolase-dye complexes, respectively, indicating that the dye does not bind to the nucleoside or sugar phosphate-binding sites on these enzymes.

These measurements predict that either the catalytic or effector sites of phosphorylase *a*, phosphofructokinase, fructose diphosphatase, aldolase, and pyruvate kinase are likely to be constructed by supersecondary structures closely resembling the NAD domain. The occurrence of such a large common element within the structures of the majority of the glycolytic enzymes suggest that such enzymes may be derived from a common ancestral protein. The predicted occurrence of a structure similar to the NAD domain, in aldolase is particularly noteworthy as this enzyme has no known nucleoside phosphate substrate or effector.

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Table 1 Interactions of glycolytic enzymes with blue dextran-Sepharose affinity columns

Enzyme	Source	Bound	Eluant
Phosphorylase <i>a</i>	RM	Yes	10 mM AMP
Phosphoglucomutase	RM	No	—
Hexokinase	Yeast	No	—
Phosphoglucose isomerase	Yeast	No	—
Phosphofructokinase	<i>Escherichia coli</i>	Yes	0.1 mM ATP
Fructose diphosphatase	RM	Yes	1 mM AMP
Aldolase	RM	Yes	10 mM FDP
Triosephosphate isomerase	RM	No	—
Glyceraldehyde-3-phosphate dehydrogenase	RM	Yes	10 mM NAD
Phosphoglycerate kinase	Yeast	Yes	1 mM ATP or 3-PGA
Phosphoglycerate mutase	RM	Yes	0.5 mM 2,3-PGA
Enolase	RM	Yes	1 mM 2-PGA or PEP
Pyruvate kinase	RM	Yes	10 mM ATP or PEP
Lactate dehydrogenase	RM	Yes	1 mM NADH

Bound, indicates whether up to 0.5 mg of enzyme binds to a blue dextran-Sepharose affinity column having a 1 ml bed volume equilibrated with 10 mM Tris-HCl buffer, pH 7.5. Eluant, indicates concentration of the listed compound added to the equilibrium buffer which elutes the bound enzyme in at least five column bed volumes.

RM, Rabbit muscle; FDP, fructose-1,6-diphosphate; PGA, phosphoglycerate; PEP, phosphoenolpyruvate. All enzymes were commercially available purified proteins except for *E. coli* phosphofructokinase which was purified as described previously¹⁸. Enzymic activity was measured by standard assay procedures.

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X-ray diffraction patterns from haemoglobin-free erythrocyte membranes

In a survey¹ of X-ray diffraction patterns from erythrocyte membrane preparations produced by hypotonic lysis within a range of tonicities and pH, we emphasised the lability of the haemoglobin-free membrane preparation which readily degenerated during dehydration to give both lipid and residual membrane (lipoprotein) phases. The lipid was identified with a diffraction periodicity of 5–5.5 nm which was sensitive to temperature changes and which could be eliminated by treatment of the membranes with phospholipase C before dehydration². In electron micrographs of sections prepared from the same samples the lipid phase was identified with regions of very fine layering (4 nm periodicity) scattered irregularly through a mass of closely packed layering of much greater dimension, which undoubtedly represented the condensed erythrocyte ghosts. These closely packed ghosts provided periodicities of 20–30 nm, each period including two apposed thicknesses of membrane which were clearly asymmetrical. The corresponding X-ray diffraction patterns also revealed this higher periodicity. We have since experimented with various conditions which can be used to provide haemoglobin-free membranes and have obtained much-improved X-ray diffraction data which confirm and extend our earlier conclusions.

Stomatoff *et al.*³ carried out X-ray diffraction studies from which they concluded that the hydrated, haemoglobin-free erythrocyte membrane is only 5.5 nm thick and is a symmetrical structure. Blaurock and Lieb, in a comment in the News and Views section of *Nature*⁴, remarked on the similarity of the electron density profile proposed for this membrane and the profile of a bimolecular layer of lipids⁵. We have no doubt that the 5.5-nm periodicity analysed by Stomatoff *et al.* does relate to a lipid phase formed in the haemoglobin-free erythrocyte ghost preparation during partial dehydration, and that they have either failed to record or overlooked the higher periodicities which relate to the membrane structure. We have studied membranes prepared exactly as reported by Stomatoff *et al.* and have found their diffraction and electron microscope characteristics to be essentially similar to those of other haemoglobin-free ghosts prepared in hypotonic conditions.

The accumulated data are being analysed in detail in an attempt to elucidate the molecular changes involved in these phase separations which occur during dehydration or partial dehydration of a wide range of membrane preparations. They will be included in a more extensive publication on this topic. Meanwhile it seems important to

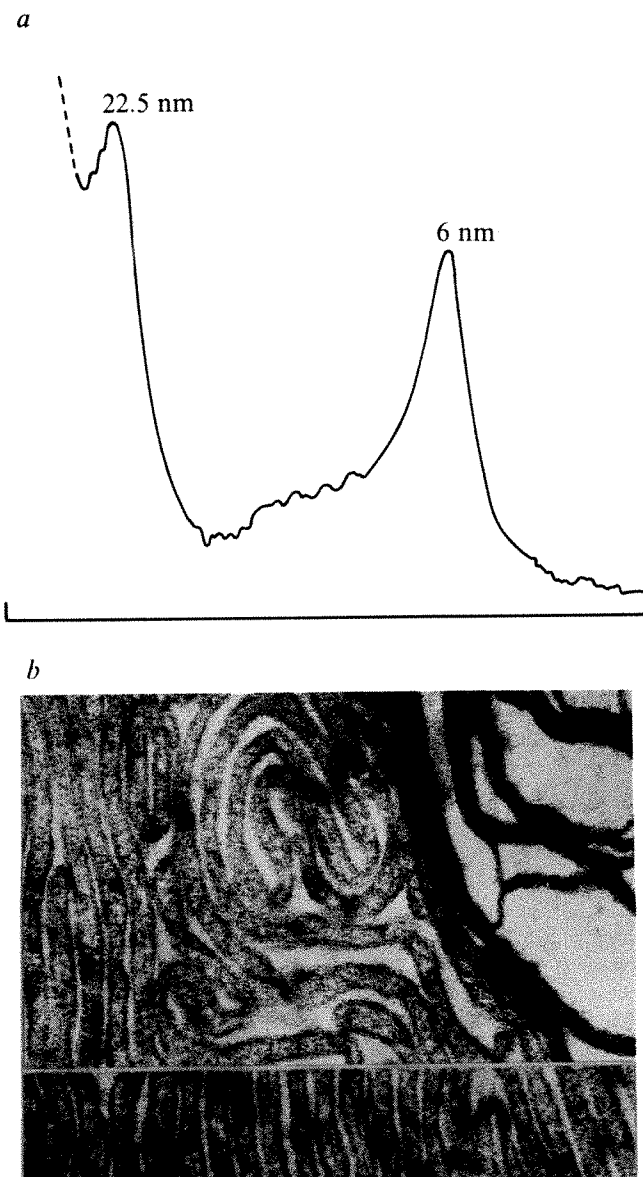


Fig. 1 *a*, Microdensitometer trace of a low angle X-ray diffraction pattern recorded from a haemoglobin-free preparation of erythrocyte ghosts (see text) after partial dehydration. The two principal diffraction bands are identified in terms of the periodicities they represent. *b*, Electron micrograph ($\times 160,000$) from the same erythrocyte membrane preparation, fixed with OsO_4 after partial dehydration, embedded and thin-sectioned. The fine layering on the right is suggested to correlate with the smaller diffraction periodicity and the packing of residual ghosts on the left and below correlates with the large diffraction periodicity.

re-emphasise the significance of this phenomenon in relation to studies of membrane structure by reporting one well-defined example which is particularly relevant to X-ray diffraction studies of haemoglobin-free erythrocyte ghosts.

Figure 1a shows a microdensitometer trace across one of a sequence of low angle X-ray diffraction patterns recorded during the slow dehydration of a sample of haemoglobin-free erythrocyte ghosts which had been prepared (at 0–4 °C) by haemolysis of pig erythrocytes in 20 mosmol bicarbonate buffer, pH 7.0, containing 1 mM EDTA and centrifuged at 200,000g for 3 h. A sample of the wet pellet was enclosed immediately in a chamber maintained at high ($\sim 100\%$) humidity and constant (10 °C) temperature. A sequence of low angle diffraction patterns (limit of resolution 25 nm) was then recorded using a single bent quartz crystal monochromator and a line view of the focus of a rotating anode X-ray generator. Initially

only broad maxima in the region of 6.5 and 10 nm were recorded but as the former intensified and improved in definition an equally intense and well defined reflection was resolved at 2.5 nm. This reduced in successive exposures to about 20 nm and the 6.5-nm periodicity to 5.5 nm in the fully dried sample.

A parallel sample was fixed with osmium tetroxide at an intermediate stage of dehydration (corresponding approximately to the stage represented by Fig. 1a) and prepared for electron microscopy. A representative micrograph is reproduced in Fig. 1b. The bulk of the sample consisted of collapsed erythrocyte ghosts, closely packed to give a periodicity of the order of 30 nm but this was interspersed with small pockets containing material which featured a very fine (~4 nm) periodicity. There is little doubt that the 5.5–6.5-nm periodicity detected by X-ray diffraction relates to this finely layered component which we have previously identified as a lipid phase. The discrepancy in dimension is a consequence of the preparative procedures required for electron microscopy⁶. The residual membranes are responsible for the higher (>20 nm) periodicities. Each membrane features a trilamellar unit with additional dense material at the cytoplasmic face, forming overall an asymmetrical unit which is approximately 10 nm thick in the dried sample. Two such units are apposed in the collapsed ghost.

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State of chlorine and potassium in human platelets and red cells

THE behaviour of excitable cells is consistent with most of their potassium and chlorine being ionised. It has been assumed that this state of affairs is also true of other types of cell. With electron microprobe analysis we have found that most chlorine in human platelets and potassium in human platelets and red cells is present not as free ions but as complexes that can be dissolved by lipid solvents. In platelets, chlorine seems to be complexed with a proteolipid. The anaesthetic halothane, in conditions that will provoke deep anaesthesia, interferes with the complexing between chlorine and the proteolipid in platelets, so that much less chlorine is extractable with lipid solvents.

Air-dried and freeze-dried platelets and red cells had a large chlorine emission peak (Fig. 1) from all parts of their cytoplasm. The microprobe has already revealed an unexpectedly large chlorine peak in muscle¹. By comparing the Cl-S peak ratio with the Cl-P peak ratio, we have attempted to estimate whether the chlorine in platelets is in the membranes or in the ground substance of cytoplasm. Although membranes are present throughout the cytoplasm of platelets, some parts contain more membranes than others. It was assumed that regions with a large phosphorus emission peak were rich in membranes containing phospholipids, for the phosphorus emission peak almost disappears when platelets are treated with chloroform-methanol. The only cytoplasmic region that gives a sub-

stantial phosphorus peak after this treatment consists of the dense bodies that contain stored ATP, ADP and inorganic pyrophosphate². The size of the sulphur emission peak was taken to indicate the thickness of cytoplasm under the beam. We found that the Cl-S ratio varies from platelet to platelet (mean 2.27, s.e. 0.07, $n = 37$) but was relatively constant with each platelet. The Cl-P ratio varied more widely (mean 2.76, s.e. 0.15, $n = 37$). Since the standard error of the Cl-S ratio is so much smaller than that of the Cl-P ratio it seems that the chlorine in platelets is in the ground substance of cytoplasm rather than in the membranes. The reason for the variation in Cl-S ratio from platelet to platelet is not clear.

Virtually all the cytoplasmic chlorine was extracted from desiccated, air-dried or freeze-dried platelets by dry 2:1 chloroform-methanol. Extraction was nearly complete after 1 min and was complete after 15 min (Fig. 1). This was the pattern for normal platelets, for those in which secretion of dense bodies had been inhibited by 0.04 mg atropine per

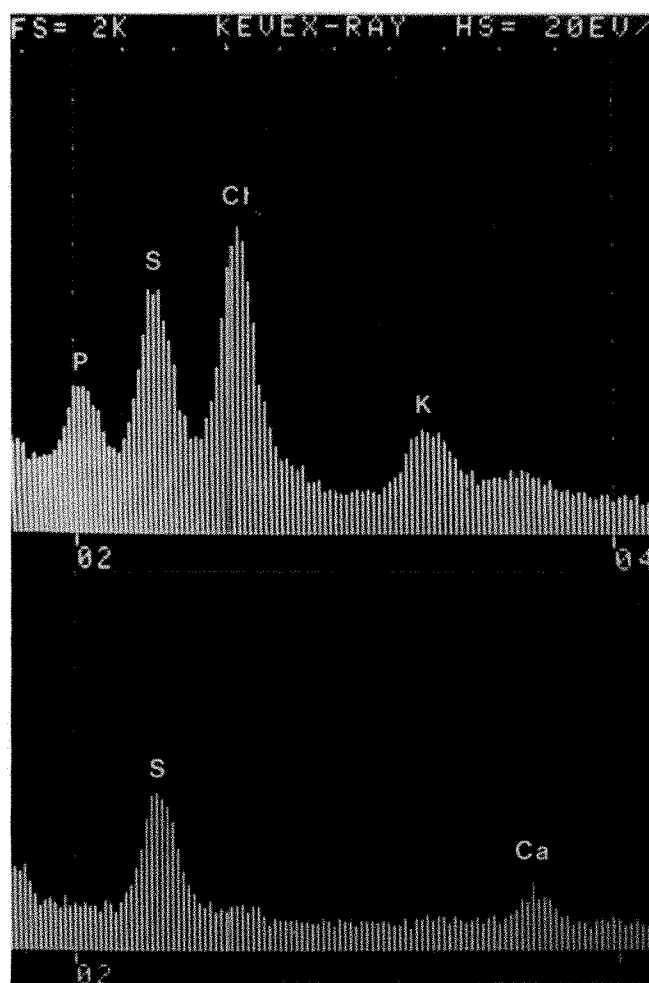


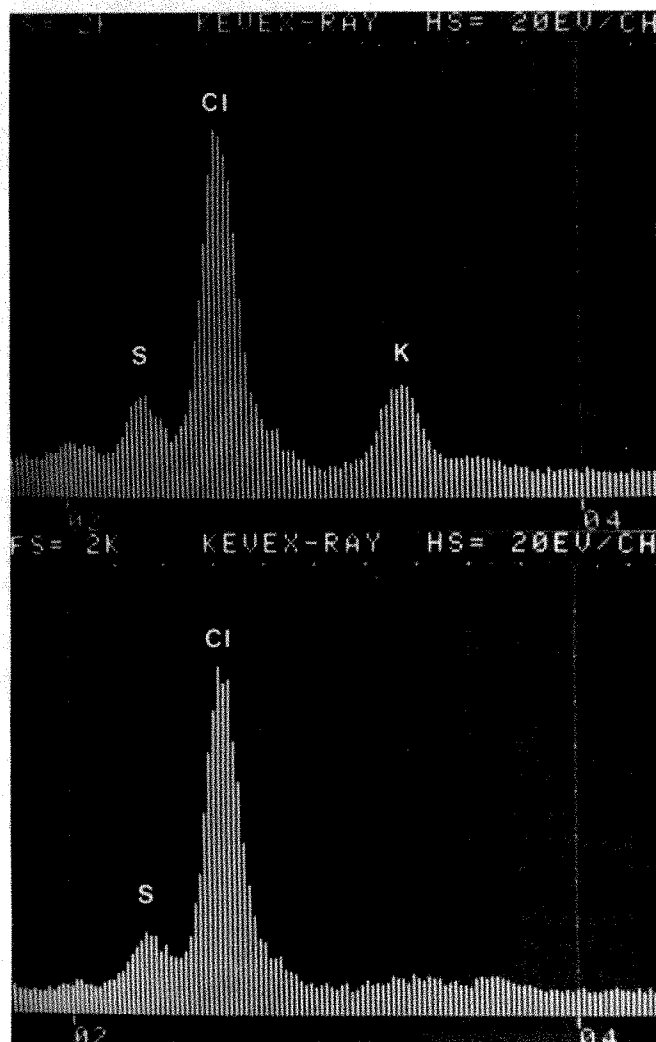
Fig. 1 Emission spectrum from human platelets. The upper spectrum is from the cytoplasm of a normal, air-dried platelet, from a region rich in potassium, the lower from a similar specimen extracted with dry chloroform-methanol. Platelets were prepared from both normal humans and pigs by placing a drop of platelet-rich plasma on carbon-coated copper grids for 10 s and blotting them. They were then either air dried and desiccated for 24 h with silica gel, or immediately freeze dried. For freeze drying the grids were immersed in liquid nitrogen and dried at -60°C in an Edwards tissue dryer Mk 1 for 2 d; they were warmed to room temperature under vacuum. Some grids were extracted for 15 min with 2:1 A R chloroform-methanol prepared from freshly opened bottles and dried with dry Union Carbide molecular sieve 4A (BDH). The specimens were then analysed in an AEI EMMA 4 electron microanalyser equipped with a Kevex Si (Li) energy dispersive detector. A liquid N_2 specimen decontaminator was used throughout this study so that the sulphur peak could be used as standard.

Table 1 Effect of treatment on chloroform-methanol extraction

Treatment applied to living cells	Extractability in chloroform-methanol after drying
Normal pig platelets	All chlorine and potassium extracted
Platelets from pigs anaesthetised with halothane	Varying amounts of chlorine and potassium retained in platelets In many platelets all chlorine and potassium is insoluble
Platelets from pigs stunned electrically at abattoir	Some chlorine is insoluble, nearly half the potassium is insoluble.

ml plasma³, and for those aggregated for 5 min by $2 \mu\text{M}$ ADP at 37°C , before drying. It seems likely that even though removal of water inevitably alters the distribution of charge within a cell, extractability of chlorine by lipid solvents is typical of these cells in life, for the following reasons. First, freeze drying should avoid the creation of high local concentrations of ions that might cause abnormal binding to cell components. No ice crystals were visible by electron microscopy in platelets freeze-dried on carbon-coated grids. Second, various semi-physiological treatments to living platelets influenced the pattern of extractability even after drying (Table 1). The variation in response to halothane was probably due to sequestration of a proportion of the total platelet population in regions where the concentration of halothane was less.

Fig. 2 Emission spectrum from human red cells. The upper spectrum is from a normal, air-dried red cell, the lower from a similar specimen extracted with dry chloroform-methanol.



The chlorine dissolved from platelets by dry 2:1 chloroform-methanol was almost all precipitated from this solution by diethyl ether. This ether precipitate consisted of white fibres, soluble, like the apoprotein of a proteolipid^{4,5} in either dry chloroform-methanol or distilled water. The ability of this complex to exist in either hydrophilic or hydrophobic situations may well be crucial to its functioning in platelets. After storage for a few days at -20°C the dry precipitate lost its solubility in water, and later in chloroform-methanol. The extractability of chlorine from dry platelets by chloroform-methanol also decreased as storage time of dry platelets increased. After prolonged storage no chlorine could be extracted from dry platelets by chloroform-methanol. In aqueous solution the ether precipitate had an absorption shoulder at 280 nm; with silver nitrate solution it gave a thick curdy precipitate shown by the microprobe to contain silver and chlorine. A balance sheet of chlorine present in platelets and recoverable from the proteolipid precipitate is being constructed.

The chlorine in human red cells was almost entirely insoluble in chloroform-methanol (Fig. 2). Red cells provided a useful internal control that demonstrated that platelets do not lose their chlorine through hydration of the chloroform-methanol. Adjacent platelets and red cells on the same grid square lost virtually all or virtually none of their chlorine respectively when extracted with chloroform-methanol.

Potassium had a very uneven distribution in the cytoplasm of platelets. Some regions had three to four times the concentration of other regions. The regions with a high concentration of potassium have not been identified. The potassium of dry platelets and red cells was almost entirely soluble in dry chloroform-methanol (Figs 1 and 2). Since this was as true for freeze-dried cells as for air-dried, this cannot have happened through abnormal binding due to high intracellular concentrations produced by air drying.

Much of the potassium extracted by chloroform-methanol was precipitated by ether as a fibrous precipitate soluble in both chloroform-methanol and in distilled water. It seems possible that, as in the case of chlorine in platelets, complexes with proteolipids influence the ionisation of potassium in red cells. This is thus a special case of the theory that cytoplasmic proteins adsorb water and metal ions cooperatively⁷. Preliminary studies with other cell types (human leukocytes, pig endothelial cells and rat striated muscle) suggest that the pattern of solubility of their cytoplasmic chlorine and potassium in lipid solvents resembles that of the red cells. The availability of free ions in cells may be controlled through complexing with proteolipids. If this were so then the observed action of halothane on platelets may be related to its anaesthetic action on excitable cells.

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matters arising

Interpretation of apparent ages in Minnesota

GOLDICH and Hedge¹ have reported ages from the Montevideo gneiss in south-western Minnesota of $3,950 \pm 70$ Myr. They also obtained an age of 3,800 Myr by including a sample from the Morton Gneiss (54 km distant). The results apparently indicate that those rocks are at least as old as any yet found on Earth. Our work in that area² does not, however, support those results. Evidence that a Rb-Sr age has been inadequately measured is provided by the failure of some of the samples to lie on an isochron (within experimental error). That occurs with two of the samples measured by Goldich and Hedge¹. In addition, the least squares fit for all of their data gave an age of $3,688 \pm 310$ Myr (2σ) and the best fit for their Montevideo Gneiss samples is $3,460 \pm 370$ Myr (2σ). In conditions of partial re-equilibration it is possible to obtain apparent ages which could be either too old or too young.

To understand better the extent of Sr mobilisation during the metamorphism of this area, we have sampled the Montevideo Gneiss on two scales: multiple samples from individual blocks about 2 m in size, and on the scale of kilometres, as was done by Goldich and Hedge¹. Analytical data are given in Table 1. The samples collected from the individual blocks may reveal local, partial or complete equilibration at the time of metamorphism. If that is so, there is no reason to believe that widely separated samples represent anything more than individual points on different local metamorphic isochrons, and their colinearity may well be fortuitous, and the age so indicated erroneous.

The Montevideo Gneiss comprises bands of grey rock interlayered with red bands. Goldich sampled the grey portions to avoid contamination with the younger red material. According to Goldich and Hedge the 3,800 Myr isochron indicates that the grey rock remained a closed system during the emplacement of the red phase (Goldich, personal communication). We do not find that to be the case. The effect of local re-equilibration is seen most clearly in the data from block MV-102 (Fig. 1a). Regardless of any original difference in age, both the grey and red bands lie on a single isochron at $2,472 \pm 44$ Myr (2σ); the high initial ratio, 0.7121 ± 0.0008 (2σ) strongly suggests that it represents a time of meta-

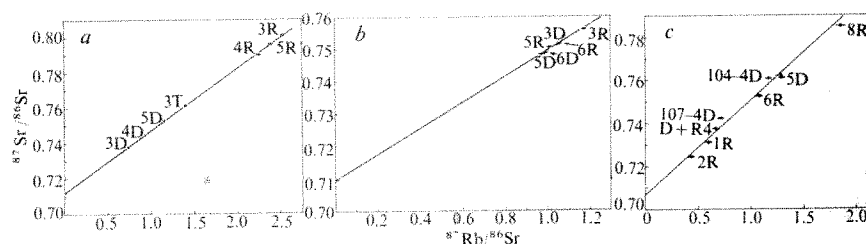


Fig. 1a Rb-Sr isochron for the red and dark bands of block MV-102 from the Montevideo Gneiss (age, $2,472 \pm 22$ Myr (1σ); initial ratio, 0.7121 ± 0.0004 (1σ). Error: 1% in $^{87}\text{Rb}/^{86}\text{Sr}$; 0.0001 (1σ) for $^{87}\text{Sr}/^{86}\text{Sr}$. b, Block MV-100 isochron with red and dark phases from the Montevideo Gneiss near Carlton Lake (age, $2,727 \pm 199$ Myr. (1σ); initial ratio, 0.711 ± 0.004 . c, Best fit isochron for samples from the locality KA-209 of Goldich¹, including the red and the dark phases (age, $3,111 \pm 202$ Myr; initial ratio, 0.706 ± 0.001 . R, Red phase; D, dark phase; T, transitional phase.

morphic re-equilibration, rather than of original crystallisation.

To a lesser but still considerable extent the effects of local mobilisation are observed at the other two sites we have sampled (MV-100 and KA-209). We believe that samples from those localities were among those used by Goldich and Hedge in constructing their 'isochron'. Locality KA-209, the designation of the most radiogenic point obtained by Goldich and Hedge, is presumably the locality previously designated that by Goldich, Hedge, and Stern³.

Results from block MV-100 are given in Fig. 1b. In this case the range of

Rb-Sr ratios is not large; however, all of the points lie well to the right of the isochron of Goldich and Hedge and, again, the dark and red rocks no longer preserve any record of possible original age differences. Samples from locality KA-209 (Fig. 1c) were not from a single block, but from an area about 20 m in dimension. Perhaps for that reason, because of local variability in the extent of remobilisation, the samples were not completely re-equilibrated at 2,500 Myr. Both the dark and red phases scatter along a $\sim 3,100$ Myr 'errorchron'⁴. Our sampling reveals no tendency for either the dark or red phases from any of these

Table 1 Analytical data

Sample no.	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{87}\text{Rb}/^{86}\text{Sr}$	Sr(p.p.m.)	Rb(p.p.m.)	K (weight %/weight)	K/Rb (weight %/weight)
a*						
4R	0.79013	2.2620	256.3	201.5	7.47	371
4D	0.74510	0.9393	258.3	84.3	2.06	244
5R	0.79634	2.4005	291.5	243.2	6.70	275
5D	0.75308	1.1576	256.5	103.2	2.36	228
3R	0.80094	2.5442	262.9	232.6	6.44	277
3T	0.76153	1.4111	249.4	122.3	1.92	157
3D	0.73764	0.73819	278.1	71.4	2.03	284
b*						
3R	0.75595	1.1705	320.9	130.6	3.18	242
3D	0.75150	1.0551	314.9	115.5	1.52	131
5R	0.75055	1.0085	329.7	115.6	2.86	247
5D	0.74835	0.9712	331.8	112.0		
6R	0.75121	1.0437	329.5	119.6		
6D	0.74876	0.9898	335.8	115.5	1.50	130
c*						
MV-9-2R	0.72456	0.4422	501.6	77.1	3.78	490
MV-9-4 D+R	0.73733	0.6662	305.4	70.7	2.39	338
MV-9-6R	0.75256	1.0826	265.8	100.1	3.19	319
MV-9-5D	0.76159	1.2994	204.3	92.3	2.55	276
MV-9-8R	0.78491	1.8580	193.0	124.1	5.45	437
MV-9-1R	0.73087	0.5840	451.3	91.6	5.07	554
MV-104-4D	0.76119	1.1696	211.7	86.0	2.51	291
MV-104-9D	0.76250	1.2918	197.3	88.6	2.42	273
MV-107-4D	0.74266	0.7180	250.6	62.5		

*a, data from MV-102 (Fig. 1a); b, data from MV-100 (Fig. 1b); c, data from KA-209 (Fig. 1c).

localities, taken individually or in combination, to be aligned on a 3,800 Myr isochron. Rather, they seem to be ancient rocks of uncertain age (probably > 3,100 Myr), which, locally, have been partially or nearly completely reset during a phase of metamorphism about 2,500 Myr BP. Ages in the vicinity of 3,300 Myr BP are suggested by zircon data^{2,3}.

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GOLDICH AND HEDGE REPLY: We wish to correct a procedural error in our original article¹ relating to the age of $3,950 \pm 70$ Myr (2σ) with an initial ratio of 0.698 ± 0.004 . The use of 2σ is not proper because of the small number (5) of samples. The recalculated results give an age of $3,950 \pm 130$ Myr with an initial ratio of 0.698 ± 0.002 (95% confidence level). We included "sample 339 from the Morton Gneiss as a guide to the initial ratio of 0.700 and an age of 3,800 Myr."

Rb-Sr analyses of the tonalitic phase of the Morton Gneiss of Lund² have progressed to a 7-point isochron with an apparent age of $3,630 \pm 60$ Myr with an initial ratio of 0.6994 ± 0.0004 (95% confidence level). The data will be published in the near future. Additional work is also in progress in the Granite Falls

area. The fact that two points do not lie on the isochron in our original diagram means that those samples have had somewhat different histories and is not evidence that an age has not been measured. In this regard some of Farhat and Wetherill's data are relevant.

Four samples of the 'red phase' from locality KA-209 and three samples from locality MV-100, together with three unpublished analyses by Hedge of the more massive granitic phase, define an isochron (Fig. 1) with an apparent age of $3,000 \pm 90$ Myr with an initial ratio of 0.7065 ± 0.0016 (95% confidence level). Three different localities are represented, but in spite of Farhat and Wetherill's reasoning we feel that the linearity is more than simply fortuitous. The 3,000-Myr age in the Granite Falls area was noted in our original paper¹. It may represent a regional high-grade metamorphic event that affected both gneissic and more massive phases of the Montevideo Gneiss, but it is possible, if not more likely, that it dates the time of intrusion of granitic magma in a foliated terrain of tonalitic to granodioritic rocks. Both phases were later affected by at least two younger events. We are dealing with a complex geological history in which the effects of interaction between magma and country rock as well as regional metamorphism must be considered.

The 2,470 Myr isochron (MV-102) also is useful, and is not unexpected as we have similar unpublished data. The Montevideo Gneiss has undergone intensive shearing and hydrothermal alteration, and some of the apparent ages in the range 1,850–2,500 Myr BP (ref. 4) may be related to this type of activity. We suggest that Farhat and Wetherill have not considered the variety and complexity of the geological processes and that neither the 3,800 Myr isochron¹ nor the 3,000 Myr isochron are necessarily 'fallacious isochrons'⁵.

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Inhibition of Na, K-activated ATPase and release of neurotransmitters

GILBERT *et al.*¹ have raised the question whether a change in the local environment of the neuronal membrane Na, K-activated ATPase (ATPase) could result in a change both in its activity and in its conformation such that an increase in exocytosis occurs; in this manner the activity of ATPase in the

nerve terminals and the regulation of neurotransmitter release would be coupled. It has also been suggested that the physiological release of acetylcholine from guinea pig ileum and rat brain cortical slices may be mediated through inhibition of the neuronal membrane ATPase^{2,3}. We have shown that several procedures which are known to inhibit ATPase cause a marked release of noradrenaline from sympathetic nerve terminals of the cat spleen^{4,5}. Since physiological release of neurotransmitters is dependent on calcium entry into the neuron^{6,7} the question arises whether calcium entering the neurone during depolarisation may inhibit ATPase⁸, so that inhibition of ATPase is the underlying mechanism in the physiological release of neurotransmitters.

It is generally believed that physiological release of noradrenaline from sympathetic nerve terminals occurs by exocytosis. This belief is mainly based on the demonstration of proportional release of noradrenaline and the soluble form of the vesicular enzyme dopamine- β -hydroxylase (DBH) in response to stimulation of sympathetic nerves⁹. If the physiological release of noradrenaline is mediated through ATPase inhibition, then procedures leading to a decrease in ATPase activity should cause a proportional release of noradrenaline and DBH. We demonstrated however that one such procedure, namely sodium deprivation, caused a pronounced release of noradrenaline (800 ± 85 ng g⁻¹, $n=3$) from cat spleen slices yet the release of DBH over the background level was barely detectable¹⁰. This result indicates that at least noradrenaline release from sympathetic nerves by sodium deprivation is due to some mechanism other than exocytosis. If the mechanism of release of noradrenaline by sodium depletion is presumed to be due to inhibition of ATPase⁴, then physiological release of neurotransmitters by exocytosis is not simply the result of ATPase inhibition.

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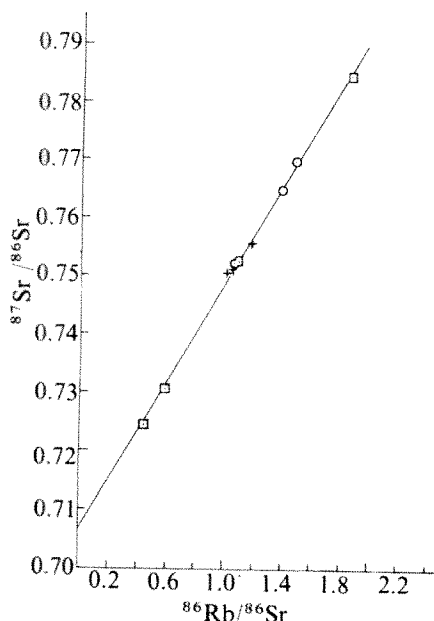
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Fig. 1 Rb-Sr isochron diagram of the massive granitic phase of the Montevideo Gneiss. Localities KA-209 (□) and MV-100 (+) from Farhat and Wetherill³; ○, new data.



reviews

AN author with the technical knowledge and presentational skill of John Maddox needs no introduction or recommendation to readers in the field of energy. His experience as science correspondent of the *Guardian*, Editor of *Nature* (1966–73), and his frequent contributions to radio and television on topical scientific subjects fit him well to project himself 'beyond the energy crisis'. Those who have read his earlier work, particularly *The Doomsday Syndrome*, will want to get hold of this new one*.

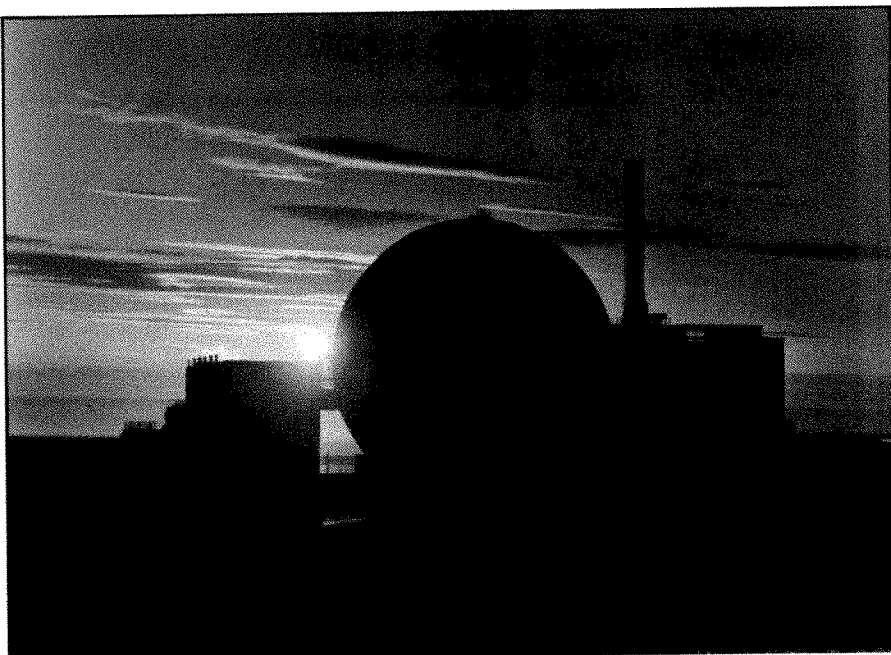
There are no visual illustrations or tables in the book but the words are well chosen and the sentences crisp and vivid. He prepares a launching pad for the main verbal discussion of energy market forces, success by the OPEC oil cartel and the problems of heavily oil-dependent customers by considering the "history" of energy supply and use.

In describing the patterns of energy consumption Maddox ensures that the reader need not be technical to enjoy and understand the presentation. He explains the units for energy and power clearly for the layman and is constantly bringing in something of interest for the specialist—for example, that the Second Law of Thermodynamics was the test advocated by C. P. Snow for the literacy of non-scientists. Both would of course know now about the heavy dependence of many countries on imported oil, but perhaps not the detail of it for specific countries; nor might they have fully appreciated the financial problems arising for many industrial and developing countries alike whose development processes are postponed when oil supply is restricted.

Maddox the realist comes through as he assesses the coal, oil, gas and nuclear energy supply prospects (the new lodes to work) and weighs their advantages and disadvantages. Why not have the best of both worlds and rely instead on new sources of energy free from hazards—solar, geothermal, thermonuclear? The short answer—serious doubts about the cost and the feasibility of meeting growing demand for energy by these means. He concludes that the contribution they will make to future energy supplies will be negligible for several decades and may always be small.

When discussing the policies fol-

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Rising to the occasion

lowed by the energy hungry, advanced or industrial countries Maddox asks why their governments should have been so naive in failing to follow the precepts of elementary economic textbooks, and concludes that they simply did not look far enough ahead to protect their interests against an oil supply cartel or shortage. He criticises their foolishness in not having seen the signs, for example, from events in Iran during the Moussadeg period 1952–54, but he is a bit unfair to say that the British decision to initiate the 1955 nuclear power programme because of the fear for the security of oil supplies was wrong. With hindsight it should have been doubled again by 1960 while sticking with the Magnox system, with which design and construction experience had been gained; and again in 1965 with only one Advanced Gas-cooled Reactor Power Station innovation being allowed as a lead into the commercial field. The early Arab-Israeli wars were a sufficient pointer to the necessary British energy strategy.

Variation in tempo is a feature of the book, especially as the international monetary problems of today are left behind. The cost of and benefit from insulating a house in Britain enters the discussion. In suggesting that it could be much better to import oil, even at OPEC prices, than to improve the efficiency of energy consumption

Maddox fails to put a value on independence from a cartel which might act again for purely political reasons.

Looking beyond the energy crisis to the coming decades the realist Maddox again emerges, seeing it as unthinkable that Britain would consider the unemployment that a sharp decrease in energy consumption would cause more acceptable than the small risks of reactor accidents; just as developing countries would not settle for slower economic growth or reduced agricultural output for the sake of keeping nuclear power at bay.

Clearly, there is an interesting way ahead, with the possibility that the oil consumers might reduce their oil consumption by 10 to 15% to bring the divergent interests of OPEC members to the surface and/or diversify their energy resource investments, or apply import duties and quotas as a means for encouraging a more equitable, lower price to be set for oil. Clearly, the energy crisis is not over; how can it be when our industrial revolution of more than a century ago is still spreading round the world with a voracious energy appetite. The oil crisis is today, the uranium crisis may be tomorrow; both are seen by Maddox as "merely occasions for the exercise of that blend of courage and realism on which industrial society is founded."

G. R. Bainbridge

* *Beyond the Energy Crisis*. By John Maddox. Pp. 208. (Hutchinson: London, 1975.) £3.95.

Plant and leaf

Phytochrome and Photomorphogenesis: An Introduction to the Photocontrol of Plant Development. By Harry Smith. Pp. xiii+235. (McGraw-Hill: London and New York, 1975.) £7.95.

THE quotation from Stephen Hales and the coloured frontispiece illustrating light- and dark-grown seedlings are a fitting introduction to the contents of this book. In the nine chapters that follow the author has analysed how light from particular spectral regions is perceived, measured by the plant and translated through modifications of biochemical pathways into a recognisable pattern of growth and differentiation. Apart from relevant discussion of the spectral activity and nature of the receptor for phototropic responses, growth effects dependent on the direction of light (phototropism) or time of exposure to light (photoperiodism) are not within the scope of this book.

Before describing the evidence for the existence of specific photoreceptor molecules the author has included a chapter on the physical properties of light in relation to action spectra and photochemical response with methods for experimental manipulation of light sources. This will be particularly help-

ful to those who are entering this field of photobiology for the first time.

The important experiments leading to the discovery, isolation and identification of a chromoprotein absorbing at red and far-red wavelengths, and the evidence for another receptor absorbing in the blue region of the spectrum, have been carefully culled from the literature, and are dealt with historically and analytically with great clarity. In one chapter the chemical properties of phytochrome are treated in detail, with present views of the likely photo-transformations and conversions of the chromophore-protein complex both *in vitro* and *in vivo*.

The quantitative detection within the plant, the likely localisation within membranes, and the evidence linking developmental responses to different forms of phytochrome *in vivo* is presented in a way that leaves the reader in no doubt of the hard facts as well as the problems and paradoxes.

Major proposals for the mechanism of phytochrome action are discussed on the basis of chemical and biological data. The extensive studies of phytochrome involvement in germination and seedling growth form the subject of two chapters, but here some crispness of presentation is lost amongst the many factual details. The longest chapter is devoted to biochemical changes associated with the phytochrome-mediated photocontrol of development.

The book as a whole is carefully and thoughtfully written and easy to read, its many subtitles providing quick reference from one section to another. As a comprehensive survey of the present state of knowledge in the field, with pointers to areas of future study, this work is valuable for students, teachers and research workers and all who wish to learn about phytochrome in plant growth. An informed interest in photomorphogenesis must surely result for anyone who reads this stimulating book. **Daphne J. Osborne**

The Shoot Apex and Leaf Growth: A Study in Quantitative Biology. By R. F. Williams. Pp. vii+256. (Cambridge University Press: London, March 1975.) £6.50; \$18.95.

THE organisation of leaf primordia at the shoot apex, which in many cases can be described mathematically, is a fundamental and obvious system for studying the concepts of positional information and development of form in plants. It is always surprising to me that the shoot apex has not been exhaustively studied in this respect, compared with the more widely used animal systems. A treatise on the shoot apex and leaf growth is therefore potentially exciting reading to those interested in the development of

form. The bulk of this book, 143 out of 223 pages, is devoted to detailed descriptions, ultimately expressed as three-dimensional scale drawings, of the vegetative growth of a dozen plant species encompassing a wide range of different developmental patterns. The development of the inflorescence of wheat is also described, and the detailed comparison of vegetative and floral apices provides some insight into the control of floral induction. These results are supplemented by a further 22 pages detailing the more complicated aspects of the methods used in these studies. After reading these sections which, in the author's words "many will be content to treat as resource material . . . to check the claims made elsewhere", the potential of the shoot apex as a system for studying the development of form is fully justified. Unfortunately, very little is concluded from this wealth of information, and the author in fact suggests that further "comparative, quantitative morphology of these objects will certainly yield valuable insights in its own right". Perhaps, however, this is the time to put the microtome back on the shelf, and try to formulate the type of "insight" on which this type of analysis may throw some light.

The major conclusion drawn from these studies is the importance of physical constraint on the growth rate of the apex and component parts. Although, with hindsight, this is perhaps an expected dependence, it is well supported by the developmental data from the different types of apex, and tested quite convincingly in a series of experiments involving tiller formation in wheat. The claim that physical constraint may play a part in the genesis of form is less convincing. Whether the physical constraint at the shoot apex is the cause or the result of the phyllotaxis and leaf shape is a moot point. It is disappointing that no attempt has been made to experimentally check this hypothesis.

Preceding these sections on the apical growth studies the book contains an interesting chapter (18 pages) on the quantitative description of growth, indicating the problems involved in analysing the growth of a complex, multicellular system. This chapter sets the scene for the subsequent relatively sophisticated growth analyses of the leaves and apex. The subject of phyllotaxis, and particularly the parameters used in quantitatively describing the phyllotactic systems, are very usefully discussed in an introductory chapter (30 pages), although I found it necessary to refer to the more detailed original treatments of Richards in one or two places.

This book is a very significant contribution in terms of the elegant descriptions and analyses of growth of the shoot apex and leaves, but where do we go from here? **John Ingle**

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Image Science: Principles, Analysis and Evaluation of Photographic-type Imaging Processes. By J. C. Dainty and R. Shaw. Pp. xiv+402. (Academic: London and New York, January 1975.) £9.80; \$26.00.

SCIENTISTS are constantly finding new ways of exploiting the immense capacity of photographic film for the recording of scientific data. This book will assist students to join in this enterprise, although it will hardly open their eyes to the modern alternatives to film. It deals with the theory of ideal and real photoreceptors of the photographic type, quantum sensitivity, densitometry and the fundamentals of the analysis of image resolution, image noise and the information content of images. The fundamental derivations are certainly given in a way which enables wider use and could be applied to electrooptic image recording and holographic methods, but the techniques themselves are not explained and are scarcely mentioned in the body of the book.

In its sphere, however, this is a good textbook, helping the reader along by its use of well-chosen diagrams and exercises.

Andrew Holmes-Siedle

Principles and Practice in Modern Archaeology. (Teach Yourself Books.) By David M. Browne. Pp. x+262+11 photographs. (Hodder and Stoughton: London, July 1975.) £1.50.

DAVID BROWNE'S new book is a welcome addition to the growing literature on practical archaeology, all the more so since it is written with both the layman and the would-be student in mind.

After a rather heavy start, defining certain archaeological concepts, the author moves easily through the more practical aspects of the discipline dealing first with techniques of discovery, then with excavation and finally with the treatment and analysis of materials from excavation and fieldwork. He writes clearly and with the good sense born of experience.

The layout is straightforward and the reference system excellent, in that it leads the interested reader immediately to the most significant up-to-date works without losing him in a flurry of superfluous detail. A list of the major museums is appended for good measure.

This book is to be highly recommended, it cannot fail to dispel the popular misconceptions which still bedevil the subject. For those taking part in excavations it should be compulsory reading.

B. W. Cunliffe

Biocontrol of Rodents. (Ecological Bulletins No. 19) Edited by Lennart Hansson and Bo Nilsson. Pp. 306. (Swedish Natural Science Research Council: Stockholm, 1975.) 45 Skr.

THIS bulletin presents the papers from the Nord-Mus symposium on rodents and forest damage held at Lammi, Finland, in 1974. The early papers review the problems clearly and reveal the severity of the situation especially in monoculture situations.

The greater part of the symposium was concerned with the various app-

Books brief

roaches to rodent control based on the increasing reluctance to add synthetic substances to the environment and the need and wish to control rodents by manipulating the qualities of the environment and the biology of the animals. In addition to being good reviews these papers are a stimulating challenge to biologists to pursue such topics as habitat manipulation, competitive displacement, plant repellence, social mechanisms and the feasibility of genetic control of small mammals. Such work will need a multidisciplinary approach and considerable resources. It is to be hoped that the effort and forward looking nature of this symposium will be rewarded with the assistance it deserves.

Graham Twigg

Engineering in Medicine. By B. McA. Sayers, S. A. V. Swanson and B. W. Watson. Pp. vii+103. (Oxford University: London, May, 1975.) £3.25.

THIS is a curious book but I am afraid it does not quite live up to the description on the cover: "a selective review. Using this as a basis, the authors then discuss prospects and priorities—addressing the problem of identifying developments that should be supported . . ."

I have great sympathy with the authors: everyone in the bioengineering field has opinions on the future of bioengineering and of bioengineers; but if the main part of the book is intended to give an authority to the views contained at the end, apparently addressed to the health departments, it does not succeed very well. I think it is unfortunate that they made the review so wide because, even when one knows the difficulties involved and accepts this explanation, the uneven treatment is still disconcerting. Their technique of condensing infor-

mation has led them to use a profusion of expressions such as "clearly", "on the other hand", and "of course" which is also unfortunate in a book of this type.

It is simply not possible to determine for whom this book has been written. It seems to have been put together as a background book for first year students but someone has thought that it was too difficult and one chapter has more than forty phrases in parenthesis in its twenty pages which give explanations in a kindly way ranging from "features of signal shape (waveforms as they are called . . .)" to "fluid outflow (mainly as urine)". This underlies the reviewer's difficulty: if one cannot determine the real aim it is difficult to judge the value; one might hazard a guess, however, that it was not particularly great. It may be, indeed, that the purpose of the book is an expression of personal opinion on scientific management but it is hard to imagine that those able to influence the organisation of research would read right through to the last chapter, which is a pity.

D. C. Simpson

Fracture of Brittle Solids. (Cambridge Solid State Science Series.) By B. R. Lawn and T. R. Wilshaw. Pp. ix+204. (Cambridge University Press: Cambridge and London, August 1975.) Boards £7.20, \$22.00; paper £3.80.

THIS excellent little book offers a comprehensive, readable, and up-to-date survey of studies of the conditions and mechanisms in the fracture of materials. Griffith's concept of energy balance provides the basis and unifying concept of the treatment. A careful consideration of the macroscopic, molecular and atomic contributions to the energy extends the validity of the concept in a most convincing way, not entirely limited by the adjective "brittle".

The authors write from the standpoint of physical metallurgy, and the strength of the book lies in its detailed and convincing treatment of the physical processes involved in fracture. Mathematical formulations based for example, on the theory of elasticity are quoted, not derived, and the book is therefore complementary to the various monographs on fracture mechanics. Much useful information is presented in diagrammatic and tabular form, with comments in the text.

The book should remain a requirement of the serious student of strength of materials for many years.

J. W. Craggs

obituary

Sir George Thomson, who died on September 17 at the age of 83, was famous for the discovery of electron diffraction. This classical experiment confirmed the hypothesis of de Broglie, and the basis of Schrödinger's wave mechanics, and it also opened the way for an important new technique, which has become an indispensable tool of solid-state physicists and others.

He shared the 1937 Nobel Prize in Physics with Davisson who, with Germer, had seen electron diffraction independently of Thomson, at almost the same time in 1927. They were led to the discovery by an accident, which they described in their fundamental paper, whereas Thomson, inspired by the new theory, knew that such an effect was likely and set out to find it. In his Cornell lectures, which describe the early experiments, he omitted, with characteristic generosity, to mention this difference.

His work depended on a full appreciation of the then new theories and of fundamental physics, and also on the skill and caution of a great experimenter. His discovery was not published until it had been confirmed by more checks and tests than many others would have thought necessary. This discovery, and the manner in which it was made, ensure him a place among the pioneers of pure physics, but he always had an eye for the applications.

He saw immediately the possibilities of electron diffraction as a tool, and over the years contributed much to its development; for this he put to good use not only his experimental skill and flair for the technical, but also his mathematical ability, proved by gaining Firsts in Parts I and II of the Mathematical Tripos. Another subject that attracted his interest was aeronautics, with which he became involved during

the First World War, and which remained a major interest for some time afterwards. He served on the Aeronautical Research Committee at the beginning of the Second World War.

In the 1930s he became interested in neutron physics, and, with his eye for applications, took up the problem of nuclear energy as soon as fission had been discovered. A committee under his chairmanship, set up on his initiative, studied the possibilities of slow-neutron fission. When it became clear that the short term possibilities depended on fast neutrons, he became chairman of the new 'MAUD' committee, whose report led to the project being given high priority in Britain, and probably also accelerated the pace of work in the United States.

After the Second World War, another fundamental field of physics with potential applications aroused his interest. He started to study plasma physics as a means of reaching the high temperatures required for a controlled fusion reaction. This hope has not yet been realised by anyone, but Thomson and his collaborators made important contributions to the early stages of the work.

He was the son of J. J. Thomson, the discoverer of the electron, the founder of a great school of physics in the Cavendish Laboratory, and a great personality. It is often difficult to follow such a father in his own profession, but 'G.P.' did not seem conscious or embarrassed by being so overshadowed. This was no doubt due to his attitude, which was completely lacking in self-consciousness, but was governed by a warm interest in other people and a concern for the essence of the problem in hand. In his many contacts with committees and with administrative problems, there were, of course, occasions when people disagreed with

him, but they were always able to respect his point of view, and the dominant image that one took away from any encounter with him was of absolute integrity, warm humanity, and frank, even blunt, talk. His action in sharing his Nobel Prize with the technician who had been involved in the discovery is characteristic not only of his decency, but of his belief in the importance of technical innovation for the progress of the subject.

One might well apply to him a comment he made when writing about his father: "He was by no means the conventional scientist guided always by logic—if indeed such a creature exists. He was guided by intuitions, not always without prejudice."

George Paget Thomson was born in 1892, educated at the Perse School and Trinity College, Cambridge, where he obtained Firsts in the Mathematical Tripos, Parts I and II, and in Natural Science Part II. His teaching at Corpus Christi College was interrupted by the First World War, when he served in France, transferring to the Royal Flying Corps. He returned to Cambridge in 1919 and was Professor of Natural Philosophy in Aberdeen from 1922 to 1930; he moved to the chair of physics in Imperial College where he remained until he became Master of Corpus in 1952. He retired from that post in 1962, but remained active in many capacities to the end. Among his numerous honours were his knighthood in 1943, the Hughes (1939) and Royal (1949) Medals of the Royal Society, the Faraday Medal (1960) and the presidency of the Institute of Physics (1958–60) and of the British Association (1960).

After the tragically early death of his wife in 1941 he always found time, among his heavy commitments, to remain close to his four children.

announcements

International meetings

December 12–17, **Water resources: water for human needs**, New Delhi (C. V. J. Varma, Indian National Committee for International Water Resources Association, Central Board of Irrigation and Power, Kasturba Gandhi Marg, New Delhi 110001, India).

November 20, **Energy transfer in photo-synthesis**, London (Dr G. S. Beddard, Davy Faraday Research Laboratory, The Royal Institution, 21 Albemarle Street, London W1X 4BS).

December 9–11, **Second international conference on electrical safety in hazardous environments**, London (Anne-Marie Cunningham-Swendell,

The Institution of Electrical Engineers, Savoy Place, London WC2R 0BL).

December 29, **Gordon Research Conference: Metals and metal binding in biology**, Santa Barbara (Dr A. M. Cruikshank, Gordon Research Conferences, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881).

nature

October 30, 1975

Hushing up Concorde

It is indisputable that Concorde is, by today's standards, a noisy plane on take-off and landing; no public relations exercise nor appeal to patriotism can allay the displeasure (at its mildest) that Concorde causes in the region of London Airport. But we are supposed to be objective scientists, and therefore not to resort to emotional phrases and subjective opinions when there exist adequate instrumental and numerical yardsticks.

The science of noise is still in a relatively primitive state, but there is general agreement between engineers, airlines and governments that the EPNdB scale (Effective Perceived Noise Decibels) is, at least for the present, an acceptable measure. This scale makes allowance for the duration of the noise but, as it is an average, obviously does not report short term fluctuations. Present noise standards at London Airport would require Concorde, weighing about 400,000 pounds on take-off, to generate no more than 107 EPNdB. Modern subsonic planes of comparable weight such as the DC-10 and L-1011 meet these standards with at least 6 dB to spare. Concorde seems, on average, to fail to meet the standards by several decibels. Figures generally indicating this have been issued both by the Department of Trade and by the Greater London Council within the past two weeks. Values on individual occasions fluctuate substantially; sometimes the noise is as much as 10 dB above the standards, whereas on 20 to 30% of flights the standards are satisfied.

Obviously, that is worrying enough in itself, but an equally major cause for concern is that such figures could have been withheld until such a ridiculously late stage in Concorde's development. It is absurd that extensive official documentation on noise appears only when planes have been painted in their owners' colours and bookings are being made for the first commercial flights, and, moreover, when the question of noise has become central for deciding whether there shall be operations into New York. Surely scientists and engineers could have made such predictions years ago.

They did. In 1972 Mr Michael Heseltine, then Minister for Aerospace, told the House of Commons the projections for 1975 noise levels for Concorde and subsonic jets. His figure of 114 EPNdB looks in hindsight about right, and did not look too offensive when placed alongside Boeing 707-320C and 747-100 figures. But what Mr Heseltine did not do—as Andrew Wilson pointed out in his book *The Concorde Fiasco* (Penguin, London, 1973)—was put Concorde's noise properly in context with the quietened versions of these noisiest subsonics. New models and retrofitted older planes were, even in 1972, achieving 6 dB reductions on the figures quoted for their noisier brothers. Between 1972 and 1975

there has been, any neighbour to an airport will testify, quite impressive noise reductions on subsonic planes. Concorde now sticks out like a sore thumb.

What then possessed those in charge of Concorde to soldier on, in the face of these depressing figures? The answer must have been a combination of immense technological momentum and confidence that regulations at London Airport would be no obstacle (though heaven knows where the plane would land) or a belief that something technological would turn up.

The only technological fix that would have been certain to reduce noise would have been to replace the Olympus engines (which are turbojets) with by-pass engines which, under names like turbofan and fan-jets, power many planes—including the supersonic Tu-144. But Olympus engines have been associated with Concorde from the very beginning, and design had been so carefully optimised around the Olympus that the idea was unthinkable. Beyond such a change, there is a very little. Talk of inexperienced pilots and improved antinoise procedures is a red herring. And devices such as the Thrust Reverser Aft and the spade silencer have not lived up to the promise that test-bed trials held out.

Blessed with hindsight, we can now see that in the early 1970s there were no well established grounds at all for believing that Concorde could meet airport regulations. This state of affairs is now confirmed just at the time that New York is looking at the noise problems.

The story has some rather unpleasant implications for the relationship between government and science and technology. Those who worked in noise were aware, years ago, that the Concorde problem was essentially insoluble, and that the chances of finding a palliative in a strictly limited time were negligible. And yet this message never got through to, or was ignored by, those who might, given a few years, have found political or administrative solutions which would have alleviated the present situation. "We couldn't write to the papers about it", one engineer told us, "quite apart from the risk of professional suicide, we knew that an immense public relations effort would be mounted to demonstrate how limited the horizons were bound to be of one man in one laboratory."

Whistle blowing is a risky enterprise, and many whistle blowers have suspect motives. But if the present Concorde problems are the result of a studied decision to override scientific and technical advice and simply to put on a brave face, perhaps one of the few good things that could come from this whole affair would be a more adequate exposure of the terms on which governments, and industries that governments encourage, use scientific advice. □



Aage Bohr, following in father's footsteps.

Why they were worth it

Peter Hodgson assesses the achievement of the physics Nobel prizewinners Bohr, Mottelson and Rainwater; and overleaf, Michael Stoker writes about the winners of the prize for medicine, Dulbecco, Temin and Baltimore.

THE physics prize this year was awarded to Aage Bohr, Ben Mottelson and James Rainwater for their fundamental work on collective motion in nuclei. Bohr is already a famous name in physics, and Aage has now received the same distinction as his father Niels, who won the Nobel Prize in 1922 for his application of quantum theory to the hydrogen atom. This is not the first time a father and son have both received the physics prize: the Braggs received it jointly in 1915 for their work on X-ray crystallography, and the Thomsons for their work on the electron, 'J.J.' for discovering it in 1906 and 'G.P.' for showing its wave nature in 1937.

Bohr and Mottelson have worked together for many years at the Niels Bohr Institute in Copenhagen, and have maintained its reputation as one of the world's leading centres for theoretical physics, while Rainwater works at Columbia University in the USA.

The work that earned them the Nobel Prize forms one of the most recent chapters in four decades of effort to understand the atomic nucleus. The early work of Rutherford and his collaborators established that there is a tiny nucleus consisting of neutrons and protons. The problem is to understand how they are bound together, and hence to explain the observed features of nuclear reactions and nuclear structure.

We can learn something about the forces between the nucleons (a term denoting either protons or neutrons) by studying the collisions of individual nucleons with each other. The forces are certainly very complicated, and it is far too difficult to calculate the properties of the nucleus directly from them. But we do know that their net effect is to hold the nucleus together so we can represent this by an overall attractive potential parametrised by a depth and a radius. Unfortunately, calculations of nuclear properties from this model showed that some of them did not agree with experiment.

This extreme single-particle model, as it was called, was superseded in 1936 by the compound nucleus model of Niels Bohr, which took full account of the way the nucleons strongly interact. According to this model, a nuclear reaction takes place firstly by the capture of the incoming particles by the nucleus, followed by a relatively long period when its energy is shared and re-shared among all the nucleons. Finally by a statistical fluctuation enough energy is concentrated on a nucleon or group of nucleons near the nuclear surface to enable it to escape.

This model worked very well and was able to account for many nuclear

reactions, until in the 1940s it was found that many properties of the nucleons show marked changes whenever the number of neutrons (or protons) was one of the so-called 'magic' numbers 2, 8, 20, 28, 50, 82, 126, . . . This pointed to some sort of shell structure, as with atomic electrons, and these numbers were shown to follow readily from a model with a term effectively depending on the orbital angular momentum and the spin of the nucleons added to the central potential already considered. This potential has energy levels, which when filled with nucleons, one in each possible state to satisfy the Pauli exclusion principle, give automatically the magic numbers. For their independent discoveries of this Maria Mayer and Johannes Jensen were awarded the Nobel prize in 1963.

It was puzzling that the compound nucleus model requires the nucleons to interact strongly, while the shell model requires that they interact so weakly that they can follow relatively undisturbed orbits in the nucleus. This paradox is understood when we realise that the Pauli exclusion principle forbids most of the collisions that would otherwise take place within the nucleus because they would lead to states that are already occupied. A particle entering the nucleus from outside, however, has much higher energy so that the final states are seldom occupied and the interaction takes place strongly. This absorption was included in the simple models by allowing the potential to become complex at higher energies.

In all this work it was assumed that the nucleus is spherical, but data began to accumulate showing that some nuclei are quite markedly deformed. This evidence came mainly from their large quadrupole moments. Closed shell nuclei, in which the neutron and proton numbers are both magic, such as ^{16}O , ^{40}Ca and ^{208}Pb , are spherical, as is to be expected from the high symmetry of the closed shells. For other nuclei, the deformation increases with the number of nucleons outside the closed shells. It is notable that the quadrupole moment is always positive immediately before a shell is filled and negative immediately after. The quadrupole moments calculated from the orbitals only of the nucleons outside the closed shells were inadequate to explain to date.

In 1950 Rainwater suggested that the extra nucleons can polarise the core so that it becomes spheroidal. Thus the shape of the nucleus results from its own stabilising forces, tending to make it spherical, and the forces from the extra-core nucleons, which tend to deform it.

If the nucleus is considered to be a liquid drop, the surface and Coulomb energies are proportional to the square of the eccentricity, for a spheroidal deformation. The effect of the distortion on the individual shell model orbits can be found by calculating the eigenvalues for a particle in a spheroidal potential, and together they give an energy that decreases linearly with the eccentricity e . Thus the total change in energy due to the distortion is

$$\Delta E \approx c_1 e^2 - c_2 e$$

and the stable shape for minimum energy is given by $e = c_2/c_1$. The constants c_1 and c_2 are known quite well, and Rainwater showed that this gives quadrupole moments that are similar to those found experimentally, and accounts for their variation with shell structure.

This vital suggestion removed the main difficulties about nuclear quadrupole moments, and provided the essential basis for a detailed theory of nuclear deformations. This was developed by Bohr and Mottelson and collaborators during the following decade. In their earlier calculations they treated the nuclear core as a charged, deformed drop of nuclear liquid interacting with the few nucleons outside the core. The motion of the core is described by a few dynamical variables, while the extra-core nucleons are treated individually. In later work they considered all the nucleons, and allowed them to move in a non-spherical potential, which represents the long range correlations between the nucleons.

Bohr and Mottelson recognised that nuclear deformations can be of two types, static and dynamic, or more simply that nuclei are either hard or soft. Hard nuclei keep their shape, but if they are deformed they can be set into rotation, like a rotating rugby football. The energy levels of such a system can be calculated quantum mechanically, and are given by the formula

$$E = \hbar^2 J(J+1)/2I$$

where I is the moment of inertia. In many nuclei, particularly the rare earths, whole series of rotational bands, each with many states, have now been identified. The theory, with its more detailed development, predicts these levels very accurately.

Soft nuclei, on the other hand, are easily given oscillations or vibrations, like the wobbling of a jelly. Calculated energy levels agree with those observed in some nuclei. The vibrational spectra are not as well marked as are the rotational spectra in hard nuclei because the vibrations are easily coupled to other types of motion, thus complicating the spectra.

One of the best known examples of nuclear collective motion is fission,



Rainwater (left) and Mottelson: prize for work on collective motion in nuclei.

when the nucleus breaks into two nearly equal parts. Niels Bohr did much of the early work on fission during the war years using the liquid drop model of the nucleus. In the 1950s Aage Bohr approached the problem from the microscopic point of view, in terms of the energy states of the fissioning nucleus. He showed that low energy fission takes place through very few reaction channels even though the level density is very high. This happens because most of the energy of the incoming neutron is spent on deforming the nucleus instead of exciting it internally. He has also made important contributions to the theory of the mass distribution of the fission fragments and of their angular distribution.

These rotational and vibrational models of the nucleus are known as collective models because unlike the independent particle or shell model for spherical nuclei they both require the nucleons to show collective or bulk motion. The nucleons are still moving rapidly along the independent shell model orbits but on a longer time scale the orbits change so that there is a resultant bulk motion of the nucleons, first in one direction and then in another, that forms the rotation or

vibration of the nucleus as a whole.

The great achievement of Bohr and Mottelson was to put all this work on nuclear collective motion on a sound and detailed quantum mechanical basis, and to apply the theories to account in detail for the properties of deformed nuclei throughout the Periodic Table. The hard nuclei are represented by a potential whose surface is expressed as a series of spherical harmonics, each with a coefficient giving a measure of the quadrupole, octupole, hexadecupole . . . deformations. The vibrations are similarly represented by dynamical deformation parameters that vary with time. All this work is described in detail in a monumental work on nuclear structure that Bohr and Mottelson have been writing for many years and which is being published in three volumes, the first of which appeared in 1969. These grew out of a series of lectures that is a continuing feature of the life of their institute. They are still full of activity, and will continue to contribute to our knowledge of nuclei, and to provide a source of stimulus and encouragement to younger workers in the years to come. □

Peter Hodgson

Nobel Prizes: 2

ALTHOUGH the pioneer discoveries on tumour viruses by Peyton Rous were begun in 1911, it is only in the past two decades that the particular importance of these viruses has been recognised, not only as causes of cancer, but as vital keys to the structure and function of animal cells. We now know that a prime feature of these tumour viruses is their ability to exist as integral parts of the chromosomes of their hosts, carried indefinitely from generation to generation in tumour cells, sometimes in normal cells, and even in germ cells, from parent to offspring. It is not surprising that the 1975 Nobel Prize for Medicine should be awarded to David Baltimore, Renato Dulbecco and Howard Temin, the three scientists whose discoveries revealed a phenomenon with such wide implications.

There can be few research workers with such a sustained harvest of discoveries as Renato Dulbecco. After graduating in medicine in Italy, and a period of initiation into phage genetics with Salvador Luria, he moved to CalTech in 1952, and during the next few years, with Marguerite Vogt, his long standing collaborator, he set the scene for the whole new era of quantitative animal virology in cultured cells which has followed. This was begun by the development of the plaque assay, a precise and direct measurement of virus particles, making possible for the first time a detailed analysis of virus growth and virus antibody interaction, to say nothing of the isolation of genetically pure stocks of virus, an essential first step for the subsequent isolation of mutants. Much of this early work was with polio virus and provided the basis for the development of the live poliomyelitis vaccines.

In the late 1950s, however, interest in Dulbecco's laboratory was shifting to the tumour viruses, and it was here that Harry Rubin and Howard Temin (then a graduate student) first successfully applied Dulbecco's quantitative approach to the study of Rous sarcoma virus, by developing an assay of transformation, a cancer-like change induced by the virus in cultured cells. Meanwhile, Dulbecco and Vogt had concentrated on the recently discovered polyoma virus from mice, and in 1959 they reported transformation by this virus leading not only to abnormal growth of cells in culture, but also to the development of tumours when the cells were transplanted into animals. In the next few years, which included a sabbatical period in Glasgow, Dulbecco and his associates then went on

to characterise the DNA which constituted the genome of the virus. It was found to be a small circular molecule, and this alone was sufficient to produce the neoplastic transformation.

The studies of transformation and tumour induction by polyoma virus, and the closely related SV40 virus of monkeys, soon posed a crucial question concerning the fate of the virus, and its role, once the initial neoplastic event had taken place in an individual cell. Did the virus simply hit and run, or did it continue to multiply and persist in all the progeny cells? If the latter, was it essential for the continued abnormal behaviour of these cells? It was soon shown by Dulbecco and Vogt that the presence and growth of virus particles were not essential features of the transformed state, but there were indications, from the work of Habel and others on new cell surface antigens for example, that viral coded information might be present in the cells. The ring structure of the viral DNA had, in fact, suggested to Dulbecco that it might be incorporated into the chromosomal DNA of the host in a manner similar to prophage in lysogenic bacteria.

It was in 1967 and 1968, after moving to the new Salk Institute as a Founder Fellow, that Dulbecco and his associates produced the first evidence that this was, indeed, the case. First with Watkins the presence of a complete set of viral genes was demonstrated in apparently virus-free transformed cells, by fusion with a second 'permissive' cell which released the block in virus growth. Then with Westphal, and later with Sambrook and Srinivasan, molecular hybridisation was used to show that viral nucleic acid sequences were not only present in the transformed cells, but were chemically attached to the chromosomal DNA of the host. This fundamental series of discoveries, and others that followed, showed how, after the initial transformation or tumour induction, the virus might persist and so affect the behaviour of all the descendants of the original cell.

To investigate the role of the tumour virus genes in the cells, Dulbecco had begun the study of conditional mutants of the viruses, first of all at CalTech with his student, Fried, who isolated the *tsa* mutant of polyoma virus, and subsequently with Vogt and Eckhart at the Salk Institute, on a whole series of new mutants. Finally, as if this were not enough, Dulbecco has produced major contributions on the physiology of the transformed cells and their normal counterparts, work which has actively continued since he moved to London in 1972.

The discovery that a DNA-containing virus could persist by integration of its genome in an animal cell was

remarkable enough, but there was at least a model available in the integrated prophage of lysogenic bacteria. The RNA-containing tumour viruses were a different kettle of fish altogether—or so it seemed. Rous sarcoma cells, or cells transformed in culture by this and similar viruses, may admittedly harbour virus particles, but since the viruses contain RNA alone there seemed to be no way in which the genetic information could be transferred into DNA to allow a stable relationship with the chromosomes of the host cells. Nevertheless, the virus-cell relationship was clearly very stable, and there were preliminary indications that some of the RNA tumour viruses could be transmitted in chromosomes from parent to offspring in Mendelian fashion. It was this problem which attracted Howard Temin.

Temin had continued his early work with Rous sarcoma virus, and using virus mutants made the interesting finding that the virus could determine cell morphology. After he left Dulbecco's laboratory he moved to the McArdle Institute at Madison, Wisconsin, and there began to study the nature of the provirus, as he called it, in transformed cells. After showing that DNA synthesis was a necessary requirement for transformation, he went on to search for viral DNA in the transformed cells. Soon came his scandalous proposal that the genetic information in tumour virus RNA was first transcribed into DNA, which was, in turn, incorporated in the transformed cells. This was not, as some have suggested, an offence to Crick's central dogma, but it was, none the less, pretty unorthodox. Indeed, when Temin's first nucleic acid hybridisation data, purporting to show Rous virus DNA sequences in transformed cells, were published in 1964, they were not very convincing and there was a good deal of sad head shaking. In time, Baluda produced more convincing evidence for viral DNA sequences in transformed cells which seemed to support Temin's views, but the principal stumbling block was the absence of any known enzyme system which could make a DNA copy from an RNA molecule, and it was this problem which Temin began to investigate.

Meanwhile, in 1968 David Baltimore had come to work in Dulbecco's laboratory. While there, and later at MIT, he made a series of important discoveries on the replicating form of the RNA of poliovirus, and on the processing of the newly synthesised virus proteins. He had not worked with tumour viruses, but was presumably influenced by the work going on around him in Dulbecco's laboratory, and by Baluda's recent hybridisation data. After he left to take up his position in

Boston, he began, like Temin, to look for an enzyme in Rous sarcoma virus which would synthesise DNA on a RNA template.

And so it turned out that, simultaneously but quite independently in May 1970, Baltimore, and Temin with his colleague, Mizutani, reported the phenomenon which soon became known as reverse transcription. In elegantly simple experiments they both showed that radioactive precursors were incorporated into DNA in Rous sarcoma virus particles containing RNA alone, and this DNA turned out to be complementary to the virus RNA. Baltimore and his colleagues have since gone on to investigate in considerable detail the activity of reverse transcriptase, the enzyme concerned, and in a series of outstanding papers have described most of the steps by which double-stranded DNA is constructed from virus RNA.

Many of the implications of the discovery of reverse transcription were

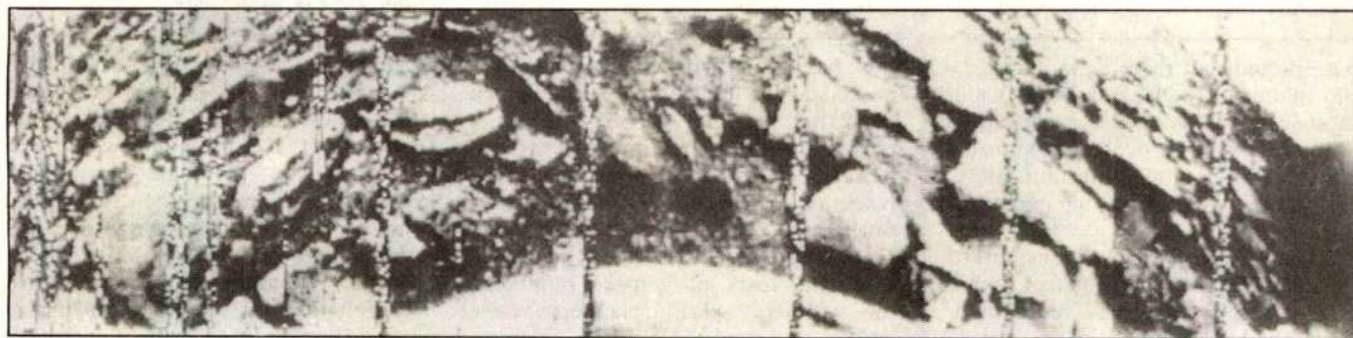
immediately obvious and were breathtaking in their scope. It immediately vindicated Temin's hypothesis that RNA tumour viruses persist in tumour cells in the form of DNA copies, and it was not long before Hill and Hillova demonstrated infectious DNA in Rous virus-transformed cells. This linked the work of Baltimore and Temin to that of Dulbecco, and permitted a unified view of tumour viruses in general, since they are clearly all DNA viruses at heart, at least in their stage of association with the host cells. In addition, the discovery of reverse transcriptase has itself provided an extraordinarily powerful new tool, since it makes possible the construction of highly labelled complementary DNA molecules which can be used as probes to search for similar sequences in either DNA or RNA. This has led, not to new and sensitive methods in the search for human tumour viruses, particularly those developed by Spiegelman, Gallo, and others, but

also to more general studies on messenger RNA and its relationship to the chromosomal DNA, for example, in studies on the haemoglobin and γ globulin genes. Finally, the demonstration that the flow of genetic information from DNA to RNA can be reversed has provoked a whole range of new and exciting speculations (and even experiments) on the relationships of viruses and their hosts, not least with Temin's own provoking ideas about the 'normal' role of proviruses in gene amplification and differentiation.

The discoveries by this year's prize-winners have opened entirely new prospects in virology and cancer studies, and have added a quite new dimension to molecular biology. Had he survived, Peyton Rous, who started it all and who waited 55 years for his Nobel Prize, would have been the first to express his delight at the Committee's choice.

M. G. P. Stoker

international news



THE two Venus probes, Venera 9 and 10, which soft-landed successfully on October 22 and 25, respectively, at sites some 2,200 km apart, have resulted in considerable rethinking of many ideas about the planet. The members of the Soviet Venus project, faced with pictures of sharp, angular, granitelike rocks (see picture above) instead of the expected sand, have been quick to comment. The sharp, "young appearance of the rocks" said mineralogist Aleksandr Vasilevskii, indicates recent seismic or volcanic activity, typical of a "live" planet. The sharp shadows, indicative of sunlight filtering through the cloud cover to the surface and the flat landscape, rather than the expected "gold-fish bowl effect" produced by postulated atmospheric distortion, have considerably opened up the possibilities of further visual observation of the surface, since it is now clear that the

Venus, courtesy of Moscow

from Vera Rich

photographs obtained can be considered and interpreted as received, without the need for empirical correction and compensation. M. Ya. Marov, of the Long-Range Space Communications Centre, spoke of the photographs as inaugurating a new era in space research, in which "clearly directed complex investigations" would replace the somewhat hit-and-miss efforts of the previous "age of reconnaissance". In the same mood of euphoria, *Pravda* also speculated on the possibility of manned Venus missions in the next century.

Meanwhile, the remaining data

gathered and transmitted by the probes, including analysis of the cloud cover and the optical characteristics of the atmosphere, has gained relatively little attention, and must await routine processing.

● The Soviet Minister of Education, Mr V. P. Elyutin, visited Britain and, more particularly, the Open University last week. This visit, clearly an exercise in *detente*, yielded little definite outcome, although some fields of cooperation between the Open University and its Soviet opposite number are envisaged. Mr Elyutin spoke of the possible mutual publication of materials, and mutual production of television programmes, and also joint research work on common problems such as environmental protection and also special research into the theoretical problems of higher education.

Speaking of Soviet higher education,

WHEN a federal court ruled last year that grant proposals submitted to the National Institutes of Health (NIH) should be made public on demand, a number of scientists, alarmed at the possibility that their research ideas could be plagiarised, went scurrying to Congress seeking a bill to override the court's decision. They have not had a very sympathetic hearing on Capitol Hill, however. It now seems that the best they can hope for is that their concern will be investigated by an independent commission.

Several scientific and academic groups, led by the Association of American Medical Colleges (AAMC) have been lobbying hard for a provision, designed to preserve the confidentiality of grant applications for at least a year after they have been funded to be attached to a bill extending the programmes of the National Heart and Lung Institute. But last week, the House of Representatives passed the bill with only a mild provision directing the President's Biomedical Research Council—an influential commission which is now examining NIH policies—to look into the matter and report by next May. A Senate subcommittee, chaired by Senator Edward Kennedy, will approve its own version of the heart and lung research bill in the next week or so,

but it is not expected to deal with the confidentiality issue at all.

It therefore seems likely that all the details contained in grant proposals will continue to be made available to anybody who goes to the NIH and asks to see them. Though NIH lawyers have

Science no secret, yet

by Colin Norman, Washington

interpreted the court's decision narrowly by insisting that it applies only to proposals which have been funded or which are up for renewal, many scientists are concerned that it could open the door to some trouble.

First, there is the problem of possible plagiarism. Grant applications are supposed to describe exactly how the proposed experiments will be carried out. The court ruling would make such details publicly available as soon as the grant is awarded, rather than when the final results are reported, and scientists are therefore concerned that their competitors could obtain complete details of what they are doing, carry out the experiments themselves, and rush into print before the hapless originator of the idea had been able to do so.

The court dismissed such notions, however, by pointing out that it makes

no difference whether or not "biomedical scientists are really a mean-spirited lot who pursue self-interest as ruthlessly as the Barbary pirates did in their own chosen field", because the law—in this case the Freedom of Information Act—clearly applies.

The AAMC is also worried that the court ruling could upset the peer-review process by which grant proposals are evaluated. The association contends that grant applicants will be reluctant to describe their proposed experiments in detail, and funding decisions will therefore be made by peer reviewers on the basis of sketchy information.

But Congress has been reluctant to step in and exempt grant applications from public disclosure for a number of reasons. For one thing, the Freedom of Information Act is an admirable piece of legislation which has ensured that much public information is actually made public, and legislators are therefore anxious not to weaken it. And for another, there has recently been considerable discussion in the United States of the ethics of various experiments on human subjects, and Congress is determined to ensure that details of such experiments are made publicly available so that ethically questionable studies cannot be hidden behind a cloak of secrecy.

he reported that there is no decrease in the number of scientists being trained in the USSR, since the proportion of each discipline in each year's turnout of students is held constant and at present the total student population is increasing.

● The Jubilee Celebrations of the Soviet Academy of Sciences, which, after a postponement of more than a year, finally took place in the first half of October, following a fairly predictable pattern.

Of far more significance was the press conference given by Dr James C. Fletcher of NASA, while in Moscow for the celebrations. As part of the Soviet-US cooperation in Space research, Dr Fletcher revealed, talks were going on to explore the possibility of exchanging ground equipment enabling each partner to monitor the weather and research satellites of the other. In addition, in exchange for data from the American Landsat satellites, which monitor natural resources, the Soviets would provide the USA with similar data from high altitude reconnaissance aircraft, which use similar techniques of spectral analysis to gather geographical data. One interesting sidelight on the proposal is that such an exchange would throw a clearer light on to the Kosmos satellite programme. Since its inception in 1962,

this has been a useful cover-all for miscellaneous objects in space which could not be otherwise explained; not only the inevitable military reconnaissance satellites, but also failed interplanetary probes, unpublished trial runs of new spacecraft and so on. Although many objects have been identified over the years by outside observers, the Soviet space planners have never commented on the identifications, although data gathered by certain named Kosmos satellites are, from time to time, published in the technical literature. If this exchange of equipment goes forward, some new designation of satellites might become necessary. Weather forecasting is already the responsibility of the 'Meteor' series. Perhaps, rather than making certain Kosmos satellites available to the Americans, a new series of geophysical satellites might be inaugurated under another name.

The postponement of the Academy celebrations from May 1974 to October 1975 was itself something of a mystery. The official explanation was that the celebrations would coincide with the Soviet elections; furthermore, that more time was necessary for the preparations at all levels of Soviet scientific society. This second reason was echoed in the press conference given on September 25 by the new President of the

Academy, Vladimir A. Kotelnikov, who spoke of "all jubilee measures" being now complete. Nevertheless, at the time of the postponement, these excuses were felt to be incomplete. Possibly the illness of the then President of the Academy, Mstislav V. Keldysh, who resigned last May, played its part. A number of observers, however, felt that the postponement might not be unconnected with the whole problem of intellectual and academic freedom in the Soviet Union, and the possible embarrassments which might be caused should visitors from abroad refer to such questions. Whatever the truth of the matter, the three dissident members of the Academy, Andrei D. Sakharov, Igor R. Shafarevich, and Veniamin G. Levich, did receive invitations for the jubilee celebrations, thus forestalling any possible criticisms on their account.

This atmosphere of goodwill was, however, short-lived. Following the announcement that Sakharov was to receive the Nobel Peace Prize, the Tass agency began a campaign of attack directed against both Sakharov and the Peace Prize adjudicators. On October 13, Levich, who had been promised an exit visa for Israel by the end of 1975, was informed that such a visa would not, in fact, be forthcoming. □

A REGULAR visitor would have been surprised: all the parking places were taken. Latecomers had squeezed their cars into corners, rolled them on to the raked gravel paths and finally let them spill down the drive. Coming to the Royal Swedish Academy of Science for the first of last week's Nobel Prize announcements was rather exciting. The second and third times were less so. It only took one visit to realise that the parking jam was not caused by eager newsmen, but by sedate Academicians meeting in traditional session; that the low murmur heard in the imposing lobby was not because the crowd was tense and suppressed, but because it was very small; and that the single flex snaking up the stairs, pregnant with promises of a media extravaganza, led in fact to the only microphone present.

Could it, realistically, have been otherwise? Like all businesses, the media invests large amounts of resources only with the prospect of a payoff. And today's Nobel Prizes for Science do not make newspapers sell. Gone are the days when the names of even scientific laureates were likely to be household words. Gone even are the days when scientists active in the same subject were certain to know of the winners. What physicist working before the mid-1950s had never heard of Fermi, Blackett or Cockcroft? Today's laureates are unlikely to be known outside their own specialisations.

This is not to question the distinction or value of the contributions made by the present prizewinners. It is merely to recognise the greatly increased scope of scientific endeavour, the pervasiveness of specialisation and the replacement of the individual scientist working in his laboratory by the team. Small wonder that the old doyens, known by all in the subject, no longer exist.

It should not really have been surprising to find so few newsmen there. The trends which have influenced the development of science have, after all, also left their mark on the reporting of it. The scope and teamwork of newsgathering has been dramatically broadened by the wire services. What role can be played by the individual pressman—be he ever so enthusiastically crouched over his telephone—in the face of the news giants whose technology will have bounced the headlines across the globe almost before he has had time to dial his number? Those few reporters who went to the Academy's announcements swapped stories about how it used to be when news sleuths concocted elaborate schemes to get the laureates' names out first: when they held lines open in local telephone boxes and waited for

the names to be communicated by pre-arranged signals from collaborators inside the Academy building—and what happened once when a man sufficiently like the key informant to be mistaken for him came out and innocently lit a cigarette, thereby putting into action an intricately planned (and later highly embarrassing) series of events.

A prize system which rewards individuals when teamwork dominates scientific research, and which rewards so few individuals when so many are now in the field, is bound to cause some

Letter from Sweden

from Wendy Barnaby, Stockholm

disenchantment. In spite, and partly because, of this, the distinction of being a science laureate is as high as ever. If the reporters' indifference is easy to understand, so also is the winners' delight.

● Sweden's ambitious nuclear energy programme is amply underpinned by the country's uranium resources, which are estimated to be the West's largest in the price range (1968 figures) of \$10–15 a pound (cheaper uranium can be mined for less than \$10 a pound). Knowing it is there is not the same as getting it out, however. The State Power Board, the government-owned nuclear energy research company AB Atomenergi and the mining firm LKAB are keen to start extracting uranium from 15 km² around South Billingen, about 350 km south-west of Stockholm. But they are not the only ones with their eyes on the area. To begin with, the region is populated and the soil is good for agriculture and forestry. Cultural historians prize it for its dozens of ancient monuments (including five gravesites dating from the Iron Age). In view of this, the military's interest seems particularly callous: they want to use it for a shooting range. And there has also been talk of the suitability of the land for limestone mining.

● Sweden and Yugoslavia are expanding their scientific cooperation. Under an agreement signed recently in Stockholm, scientists and experts from the two countries will exchange visits of 10 person-months a year, symposia will be held and the traffic of scientific and technical journals enlivened. A spokesman for the Royal Academy of Engineering Sciences, which negotiated the agreement, said that the specific areas of cooperation had not yet been decided but that they would be in applied rather than basic science.

The agreement is similar to those Sweden has with other East European countries in that, although each sig-

natory can nominate scientists it would like to host, the final decision rests with the other's authorities. In this sense it is a less exciting arrangement than that concluded this year between the Swedes and the Russians, in which each undertook to send the specific experts requested by the other. In practice, of course, this aspect of the undertaking may make very little difference to the work actually done. For the moment, it is enough that each side wants the volume of this work to increase.

● Asbestos, and its use in working environments, is the subject of new regulations issued by the Swedish Work Protection Board. In the face of increased consciousness of the dangers of exposure to asbestos dust, the victims of which at present number about 200 (including some cases of lung cancer), the new regulations aim to minimise its use, to regulate unavoidable use and to educate the users to the dangers involved.

Sweden uses about 30,000 tons of asbestos annually, half of it raw and half in the form of ready-made products such as asbestos cement. Although its use is being discontinued where substitutes can be found—as in the manufacture of torpedo boats—it seems difficult to replace it totally with safe materials. Brake linings, for example, still depend on asbestos to withstand great heat. Given this situation, total protection of the working population from this particular environmental hazard still looks to be some way off.

● Taking measures to improve working conditions is not Sweden's only activity in this area. "The working environment" is one of the obligatory subjects for students beginning university-level education in trade techniques. This autumn, eight trial courses in the techniques of the clothing, food, paper, forestry, workshop and steel industries are being held around Sweden for people who have had basic training and at least four years of employment in one of these fields. The idea is to educate experienced workmen for more responsible positions—vocational trainers, production and control technicians, foremen—within the various industries.

At Eskilstuna, to the west of Stockholm, 30 employees from different workshops—among them, sheet-metal workers and tool-makers—are starting a course that will demand 40 hours a week for three terms. Besides "the working environment", they are taking off with six subjects: Swedish, physics, mathematics, materials, drawing and organisation. There seems to be no uncertainty about their employment prospects after the course. □

THE Arab League Educational, Cultural and Scientific Organisation (ALECSO) and the United Nations Environment Programme (UNEP) have started a Regreening of Arab Deserts Programme. Task forces of consultants have visited Arab countries to interview leaders of scientific projects, heads of government departments concerned with land reclamation plans, and institutes of research concerned with desert studies and with aspects of land use in arid territories.

Their aims are to identify national experiments for applying science and technology to the fight against desert creep and to the reclamation of desert lands, the conservation of surface deposits, the use of saline water for irrigation, the improvement of technologies for 'subsurfacing' with special materials including asphalt and asphalt-like substances, the conservation of natural pasture and experimental range management, animal breeding for improved stock, sand dune stabilisation, farming in desert oases (underground water resources) and to the integrated development of natural resources and nomads.

A coordinated intergovernmental research programme, in which the Arab countries will participate, will use the information and data provided by the task forces as a basis for consultations on the scientific content, plan of action and budget of the programme. The aim is the rational use of scientific knowledge and technical innovations in halting desert creep which is now proceeding at an alarming rate, and regreening extensive tracts of man-made deserts. Also planned are a study of human impact on nature and natural resources in Arab countries and a survey of the existing national institutional arrangements dealing with deserts.

Task forces have already prepared interim reports on their visits. In Libya they found that trees planted in open sand have little chance of survival because the movement of the sand by the wind in summer, when the soil is dry, either buries them, uncovers the roots or sandblasts them. So temporary stabilisation is carried out by spraying with a petroleum mulch heated to a temperature of 40–60 °C. The cost of spraying 1 hectare is about 120 Libyan pounds (\$375).

In Saudi Arabia, a number of nurseries have been established on a total area of about 18 acres, producing 600,000 saplings a year (comprising the native *Tamarix gallica* and *T. aphylla*, and such introduced species as *Eucalyptus camaldulensis*). The result was that sand dune encroachment from the north and north-east was checked and 14 villages menaced by sand dunes have been protected. Afforestation amounts

to 1,250 acres (10 million trees) and other projects include the drainage of swamps and water-logged ground, and the establishment of a national park.

A land reclamation project has been started at Taourgha, 200 km east of Tripoli and about 25 km south of the Libyan coast. Irrigation is by artesian water from the Taourgha spring, which flows freely at the rate of 3.0–3.25 m³ s⁻¹ or about 250,000 m³ d⁻¹ and contains about 2,500–3,000 p.p.m. of soluble salts (that is, class 4 water, with very high salinity). The total area involved in the project is 3,000 hectares,

The Arab World

from Salah Galal, Cairo

and the growth of crops (alfalfa and barley) has been quite satisfactory with water containing 3,000 p.p.m. of dissolved salts. The soil is quite permeable, the drainage is adequate, and irrigation water is applied in excess to provide considerable leaching (50% leaching requirement).

In Tunisia the government established in 1962, with assistance from the UN Development Programme and UNESCO, a Research Centre for the Utilisation of Saline Water Irrigation (CRUESI) to study the prospects of irrigation with saline water and to train specialists and technicians. CRUESI has six field stations representing a range of soil conditions, water quality and climate, and has in hand a series of well designed experiments combining various water quantities, soil treatments, land preparation and crops. Tested crops included alfalfa, clover, maize, beans, pimientos, rye grass, watermelons, sugarbeet, artichokes, fodder sorghum, tomatoes and cotton. The experiments carried out since 1962 have already produced valuable scientific information.

The ALECSO/UNEP task force of consultants has recommended a co-ordinated research programme to restore and manage rangelands in Arab countries, with studies on the recovery and succession of vegetation types and changes in biomass and production with regard to grazing; selection of useful native species from the point of view of their occurrence, phenomenological development and ecological relationship; the improvement of practices like range pitting and contour furrowing; studies to control desert creeping and sand dune shifting (by such methods as mulching, wind breakers and the placing of brush barriers); wind strip cropping control; and the limitation of soil erosion by wind and water.

● Professor Mahmoud Mohamed, professor of radiotherapy and nuclear

medicine at Cairo University and ex-Minister of Public Health in Egypt, has put forward a strategy for cancer control in developing countries, with Egypt as a model. It stresses the inadequacy of basic and central health information and documentation centres (which means that assessing health problems is usually a matter of empirical estimation), the inadequacy of resources and investments for upgrading existing services and research so that the attention of the health administration is usually focused on curative rather than on preventive services; and the lag which usually occurs between health development schemes and manpower development. Suprastructure development in cancer research is usually not planned or linked to existing health priorities, says Professor Mohamed, and furthermore, it suffers from factors as diverse as a brain drain, low socio-economic standards, and illiteracy—a pertinent feature of underdevelopment which impedes the extension of health education. Short life expectancy and high infant mortality rates add to the problems of cancer control in Egypt, with the real incidence and mortality rate still unknown and information available only in the form of relative frequency data. The estimated number of new cases each year is about 10,000–12,000 (Cairo University Hospital 200; Cancer Institute 2,000; Alexandria University Hospitals 1,500; Ain Shams University Hospitals 300; Mansoura University Hospitals 200; Tanta University Hospital 530; Assiut University Hospitals 120; Ministry of Public Health Hospitals 500; private cases 3,800.)

The most common cancer, in the male, is that of the urinary bladder (21–24%) and the most common in the female is cancer of the breast (20% of all female cancer cases attending the radiotherapy department of the Cairo University Hospitals). Most patients present themselves in the late stages of their disease to the 10 treating centres (all of which are teaching centres) or to 40 general hospitals and 159 district hospitals with inadequate or old equipment.

The proposed strategy includes decentralisation of the diagnosis of cancer; centralisation of treatment, manpower development and research in university centres; relying on the GP and his team for early detection; concentrating research resources on epidemiological aspects as a priority; establishing the registration of cases as an integral part of a general health registry; and the establishment of cancer control teams in teaching hospitals.

● UNESCO plans to convene a Conference of Ministers in Arab States Responsible for the Application of

Competition 2. Limericks to illustrate scientific principles. An enormous crop; we haven't verified that the selection that we print are original, and in at least one case the science is wrong (no prizes for pointing out errors). £10 to G. J. S. Ross of Harpenden, Hertfordshire; a clear winner with black holes, and also runner up with plate tectonics.

A pedantic astronomer said—gravity
Is causing semantic depravity.

These stars may be black
As light outflow they lack,
But holes? there's no room for a cavity.

G. EDWARDS

There once were two frogs of Gondwana
Who vowed ne'er to part till Nirvana,
But each met its fate

On a separate plate:
He lies in Brazil, she in Ghana.

G. J. S. ROSS

"Apollo to Mission Control—
We are almost in reach of our goal,
But our readings of G
Seem excessive to me,
So we may be inside a black ho . . .

G. J. S. ROSS

Poor John could not savor a rose
Despite hard proboscis blows
"My genes", explained he,
"Have an A for a G
So I suffer a code in the nose."

A. MEHLER



A plumber by name of Fred Slaughter,
With a wife and extravagant daughter,
Shouted, "Praise and acclaim
For whatever's to blame
For the anomalous expansion of water."

I. P. FREEMAN

A Greek once, in Physics a seeker,
Exultantly shouted "Eureka!"

He leapt from his bath
And rushed down the path—
Archimedes, the prototype streaker.

J. N. F. JURITZ

Though *coli* may bother and vex us,
It's hard to believe they outsex us.
They accomplish seduction
By viral transduction,
Foregoing the joys of amplexus.

S. GILBERT

While searching Mt. Aetna for data,
Old Pliny once tripped on a crater.
His feet couldn't hold.

Down the mountain he rolled
At a speed nearly (g) (sine of theta).

S. GILBERT

Competition No 3

Archimedes is said to have discovered his most cherished principle in the course of messing up the bathroom floor, Newton upon being disturbed by a passing apple. Fleming left the window open and came home to penicillin. Readers are asked to provide a fictitious account (short) of similar momentous discoveries which were happened upon by chance. If stuck for topics, they might try their teeth on pulsars, double helices or good old continental drift. Closes (Dec. 5). □

Science and Technology to Development (CASTARAB) in 1976, to be organised with the cooperation of ALECSO and the Economic Commission for West Asia (ECWA).

As a first step in preparation for the conference, a preparatory meeting of experts was held in Kuwait to advise the Director General of UNESCO on the preparation of the agenda and the documents for the CASTARAB conference.

It finally recommended three main themes for the conference: trends in the national science and technology policies of Arab states; regional co-operative research projects relating to natural resources, energy, food resources and environmental quality; and integration mechanisms for science and technology in the Arab states.

● Saudi Arabia has invited Egypt to make use of the Centre for Applied Geology (CAG) which had been established in Jeddah, close to the Arabian shield (which is rich in ores and minerals). The CAG is at present offering courses in mineral exploration, hydrogeology and engineering geology. It offers the degree of Master of Science and has a two-year geology technician training programme leading to a technical diploma.

The centre also offers an opportunity for geologists from Saudi Arabia and neighbouring countries to be trained, and students obtaining their master's degree with outstanding grades may be granted a government scholarship for completing their studies for a PhD abroad.

● A study on the brain drain of physicians in Egypt shows that the main concentration in the Egyptian population is in the Nile Valley and its Delta, where the limited amount of land space means that about 99% of the total manpower is crowded into about 3.5% of the total surface area of the country. Consequently there is a regular redistribution of manpower and Egyptian physicians tend to migrate (both internationally and intranationally) with the UK and the USA proving the most attractive destinations.

Apparently, African countries prefer Egyptian physicians, with the result that there is a migration of medical students from Arab and African countries to the Faculty of Medicine at Cairo University. This year the main exporting countries for medical students were Saudi Arabia (297), Libya (181), Palestine (134), Jordan (121), Sudan (114), Kuwait (102), Lebanon (88) and other Arab countries (278)—a total of 1,315 from Arab countries; they also came from South Africa (59), Malaysia (48), Nigeria (26), other African countries (41), from Pakistan (25), and other countries (54) (making a grand total of 1,568 foreign students).

Statistics show that physicians left jobs in the rural areas at the following rates: 1972 (5%), 1973 (4.2%), 1974 (5.8%); and the number of Egyptian physicians working abroad were spread in the following way: in Arab and African countries in 1965, 269 (76.3%); in African countries 23 (6.4%); and in other countries 61 (17.3%). □

Carcinogenic agent?

A SOLVENT used extensively in academic and industrial laboratories may be a potent carcinogen when its vapours are inhaled, according to tests carried out by scientists at the DuPont corporation in Wilmington, Delaware.

Known as hexamethylphosphoramide (HMPA), the solvent has long been recognised as acutely toxic and as a skin irritant, but there had been no previous indication that it is carcinogenic. The DuPont study found, however, that rats developed a rare nasal cancer when they inhaled small amounts of HMPA for 6 to 8 months. Although the study has not yet been completed, Dr J. A. Zapp, Jr, Director of DuPont's Haskell Laboratory for Toxicology and Industrial Medicine, wrote a letter to *Nature* to "urge everyone using HMPA to handle it with the precautions appropriate to a potential carcinogen".

The experiment, which was started in December last year, involved exposing rats to amounts of HMPA ranging from 0 to 4,000 parts per 10⁶ for 6 hours a day, 6 days a week—in other words for periods of time corresponding to occupational exposure. After 8 months, some of the rats developed enlarged noses and had difficulty in breathing, and it turned out that they were suffering from a form of nasal carcinoma. "There appears to be no doubt", Zapp said, "that this rare form of cancer has been produced . . . by inhalation exposure to HMPA at a concentration as low as 400 p.p.b.". □

correspondence

EEC directives

SIR,—In his article "A community of interests" (October 16), Lord Ashby describes as "phoney" the EEC argument that to permit lower effluent standards to a Scottish wood pulp mill than to one situated on the Rhine constitutes unfair competition.

The EEC argument is valid since the clear intention of the 1973 Declaration is to remove the question of pollution control from the realm in which market forces can operate. The situation is analogous to that of safety regulations inside the suggested wood pulp mills. Such regulations are recognised as imposing a prior cost on the production process. Society, through the legislative process, has opted for such. Price competition can begin after these legislated costs have been paid—and a company can only stay in business if its rivals are obliged to do the same.

B. A. MARDALL

London, UK

SIR,—Regarding Lord Ashby's article on EEC directives and pollution, there is surely now real evidence that sewage in seawater around beaches is dangerous to health. I refer to work by Dr H. Williams Smith, a bacteriologist, who analysed samples of seawater from fifteen beaches in England and Wales, and found high concentrations of *E. coli* which had clearly got there from sewage. As Dr Smith pointed out in 1971, this could lead to *E. coli* (which are themselves harmless) being swallowed while swimming and passing on the property of resistance to antibiotics to bacteria in the swimmer's gut.

JOHN NEWELL

BBC, London, UK

The science of astrology?

SIR,—Although one can deplore the mumbo-jumbo of astrology and its patently false postulate that planetary and stellar positions are relevant to the human condition, the condemnation of it in immoderate terms by 186 scientists (September 18) could well prove equally deplorable. It should be remembered that modern chemistry springs from alchemy, beside which astrology seems almost rational!

Perhaps astrology, like alchemy, represents a corpus of observational data collected over millennia which has not yet been codified. It is entirely credible

that a foetus conceived in winter will differ (on account of maternal stress from the environment) from one conceived in summer, and the difference might have behavioural implications. If so, astrology could prove to be a repository of data, access to which is attained by what is virtually an elaborate calendar.

Anthropological or social-science studies along these lines might be profitable; certainly without them outright condemnation is rash.

S. SMITH

Epsom, UK

Botanical decline

SIR,—The declining interest in the botanical sciences is causing concern to biologists, and especially plant scientists all over the world. The implications of declining interest in plants are far reaching, particularly when the need for increasing food supplies becomes more and more urgent. It is therefore of interest to see how the plant sciences are represented in a general science journal such as *Nature*. Without claiming to have made a statistically significant study, I think the following figures are striking indeed, I counted the biologically oriented Letters to *Nature* in 10 recent issues of *Nature*. In 10 issues there were 236 such communications. Of these, 14 dealt with subjects which may be regarded as botanical in the wide sense of the word. Of these 14, two dealt with photosynthesis, three with plant biochemistry and four with fungal metabolism. In other words, the entire area of plant sciences is represented by not quite 6% of the letters to *Nature*. The results of this are of course obvious. Plant scientists being aware of the few botanically oriented articles in *Nature* will respond in two ways. They will tend not to read *Nature*, a fact easily confirmed by casual consultation of colleagues. In addition they will tend not to send papers to *Nature*, because they will readily be overlooked by those most interested.

The existence of fashions in science is well known. What is perhaps not always appreciated is just how invidious the effects of such fashions are. A fashion will lead to increased publication, which in turn draws increasingly the attention of those not working on well defined, clearly oriented problems. Such new criteria as citation indexes aggravate this. One should also

not ignore the repercussions which publication policies have on the availability of research funds. The problem of fashion exists, of course, not only in general science journals but also in specialised ones. Very often the disastrous results are not due to any deliberate editorial policy but to a snowball effect. Articles in a fashionable field will lead to an increasing volume of articles in the same area. It may be assumed that the number of good articles will be greater, the great the number of articles submitted (although this will certainly not be a linear relation). It is then inevitable that articles on fashionable subjects will flood the journals and decrease the interest in less fashionable subjects.

A. M. MAYER

The Hebrew University of Jerusalem, Israel

Cats' eyes: a new twist?

SIR,—Dr Blakemore's work on the rotation of kitten's eyes has—quite rightly—raised a storm of protest and we in the RSPCA are obviously extremely concerned at any suggestion that physical or psychological suffering has been inflicted, to whatever degree.

What also concerns us is that we seem to have yet another example of unnecessary experimentation on living animals. Work by Stratton in 1897 was developed by Snyder and Pronko (1950) and others, leading to that published by Held and Baur in 1967 and subsequently.

It is now well established that no adaption occurs in the primary visual cortex when there is rotation of the visual image; and that visual perception involves the integration of both visual and non-visual activity by the higher centres.

In view of this all that Blakemore seems to have done is to have established that rotation of the retina produces similar effects to those produced by using prisms.

Why does he not admit that this was simply another ill-advised academic exercise instead of attempting 'post hoc' justifications of his 'experiments' on the ground that they will contribute to the treatment of various afflictions which are either incredibly rare, readily studied in humans or both.

D. A. PATERSON

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news and views

A TECHNICAL Conference on Tropical Moist Forests (rainforests in the demotic) was to have been held in Brazil this September. The conference, organised by the Food and Agriculture Organization (FAO) of the United Nations, was cancelled in June 1975. With tropical rainforests everywhere under increasing pressure from human activity, this cancellation is doubly unfortunate: on a technical level it retards the dissemination of knowledge, and on a symbolic level it suggests unconcern.

Earlier ecological theory frequently held that complexity, in the sense of many species and a rich web of interlocking relationships, tended to confer stability on a community. More recent work, both empirical and theoretical, suggests that complex ecosystems (of which the rainforest is the archetype) are dynamically fragile. Although well adapted to persist in the relatively predictable environment in which they have evolved, tropical rainforests are likely to be much less resistant to the disturbances wrought by man than are relatively simple and robust temperate ecosystems. Much evidence and thinking in support of this view comes from the report of the (US) Institute of Ecology (TIE) entitled *Fragile Ecosystems: Evaluation of Research and Applications in the Neotropics* (edit. by Farnsworth and Golley, Springer, Berlin, 1974), and from the First International Congress on Ecology held in 1974 (see *Nature*, **251**, 376–377; 1974).

Such generalisations about entire ecosystems can be related to the life history strategies of the constituent species, which may usefully be discussed in terms of the deliberately oversimplified concept of *r* selection and *K* selection. This general notion goes back to Schmalhausen, Simpson, Stebbins and others, but it was MacArthur and Wilson who coined the phrase “*r* and *K* selection”, derived from the conventional parameters in the logistic equation, $dN/dt = rN(1-N/K)$. This equation describes the familiar, sigmoid curve of ultimately bounded population growth: at low population densities, there is essentially pure exponential growth, at the rate *r*; at high densities the population stabilises at a value *K* which is set by the environmental carrying capacity. A *K*-selected organism sees its environment as stable and predictable (and con-

The tropical rainforest

from Robert M. May

sequently the population is usually around its equilibrium value, $N \approx K$). The evolutionary pressures on an organism in these circumstances are, crudely, to be a good competitor; to increase its effective value of *K*; to have fewer offspring but invest more time and energy in raising them. Conversely, an *r*-selected organism sees its environment as unstable and unpredictable (and is usually at low population values, growing exponentially, and undergoing episodes of boom and bust). The evolutionary pressures here are for opportunism; for large *r* to exploit the transient good times; to have many offspring, few of which can expect to mature. Of course, reality is more complicated than this, and the paradigms of *r* and *K* selection are no more than opposite ends of what in fact is a continuum.

Notice that it is difficult to produce extinction in *r*-selected organisms, such as crop pests or the fire ant; their primary adaptation is to bouncing back from low population values. Conversely, *K*-selected organisms are geared to maintain the population around its equilibrium value, and may have difficulty in recovering from a severe disturbance. The extinct passenger pigeon is an example of a once abundant organism of a *K*-selected kind.

Broadly speaking, the contrast between the relatively predictable and stable tropical environment and the relatively vagarious temperate one can be viewed as tending to produce a *K* selection/*r* selection contrast. One manifestation of this is that ecologically analogous animals tend to give birth to fewer progeny in the tropics than in the temperate and boreal zones. Examples are agouti and paca compared with rabbits, peccaries with wild pigs, curassows and guans with pheasants and quails. Lack, Cody and others have presented a wealth of evidence that birds tend to have smaller clutch sizes in the tropics; this and other such data have been reviewed by Southwood *et al.* (*Am. Nat.*, **108**, 791–804; 1974). The overall effect is that tropical animals tend to have less capacity for the sort

of explosive, “pioneer” population growth which characterises their higher-latitude cousins (mythologised by lemmings). They are not well adapted to recovery from bad times.

There is a corresponding tendency to select for competitive ability, rather than weed-like opportunism and adaptability, in tropical plants. One consequence is that seeds tend to have little or no dormancy period, being adjusted to germination in the shady, moist, cool environment of the forest. This is in striking contrast to the wide variety of dormancy periods and survival strategies used by, say, desert plants. Thus, for example, members of the Dipterocarpaceae, the main timbertree family of South-East Asia, have seeds adapted to germinate in the stable microclimate (23–26 °C) of the rainforest soil. Clearing results in severe heating of the soil, and seedling death. The inability of these and many other seeds to recolonise, once sufficiently large areas are disturbed has led Gomez-Pompa *et al.* (*Science*, **177**, 762–765; 1972) to label the tropical rainforest a ‘nonrenewable resource’. Management of rainforests for a sustained yield timber industry is likely to require more sophisticated techniques than for temperate forests, a point underlined in the forestry conference report in *Nature* (**255**, 578; 1975).

Another relevant fact is that the sustained higher temperatures and high humidity in the tropical soil micro-environment makes for much faster decomposition rates than at higher latitudes. The typical turnover time for the decomposition of leaf litter in the tropical rainforest is 6 weeks, compared with 1 year in temperate deciduous forests, 7 years in boreal conifer forests, and 3 years in temperate grassland (Whittaker, *Communities and Ecosystems*, second ed., 248; Macmillan, New York, 1975). Tropical forests tend to respond by storing more of the nutrient pool in the plant biomass, and less in the soil, relative to temperate and boreal forests. This tends to protect lateritic tropical soils from weathering and washout of minerals and nitrates; removal of forests, as in efforts to expand agricultural production, tends to quicken laterisation, which in turn impairs agriculture.

These differences, in the tropics, between soil types which range from volcanic to lateritic, mean that un-

planned land occupation can have unfortunate outcomes. Summarising such problems in South-East Asia, Meijer (*Bioscience*, **23**, 528-533; 1973) has written: "In Vietnam, Thailand, Malaya, Sumatra, Java and the Philippines, only those areas where local agriculture is built around irrigated rice fields can have a stable agriculture and a dense population living in harmony with the environment. The vast hilly sandstone regions of Sumatra and Borneo are not suitable for continuous short-term crops, hill rice or corn; it is, in the long run, economically more productive to keep them as Forest Reserves harvested on a sustained yield basis". Eckholm's review (*Science*, **189**, 764-770; 1975) of the deterioration of mountain environments stresses the consequent effects on the lowlands, and particularly on

tropical lowlands: "The eastward movement of settlers to the jungles of the upper Amazon Basin can appropriately be labelled anarchic.—The frequent result is double disaster: a costly depletion of lumber resources as trees are cleared without regard to the soil's suitability for agriculture, followed shortly thereafter by the abandonment of the unproductive forms".

With most of the Top Ten human population growth rates currently attained in equatorial countries, an acceleration of destructive impacts on tropical rainforests is inevitable. But the long-term effect of this impact could be mitigated by coherent and well informed planning and management. It seems more likely that, in a decade or two, it will be possible to organise a retrospective conference on Tropical Moist Forests. □

Organisation of the mammalian tectum

from Shin-Ho Chung

A NUMBER of recent anatomical and physiological studies on the mammalian tectum (or superior colliculus) provide a glimpse of the ways in which the brain processes sensory inputs to make a final motor command. Axons carrying visual, auditory and somatosensory information converge on the tectum and interlace with tectal neurones, forming an intricate network of nerve cells, the main output of the tectum is then relayed to a number of motor nuclei that control the movement of the eyes or the head towards the source of stimuli. In a sense, the tectum is a microcosm of the entire brain, because it serves as a correlation centre for incoming messages and a command post for eliciting behavioural responses. For this reason, it has fascinated neurobiologists for several decades

In the vertebrates below mammals, the tectal and subtectal areas are the main centres of termination of sensory pathways. In the course of phylogeny, the tectal areas become progressively less important, and some of their functions are assumed by the diencephalon and the cortical centres. The relative size of the tectum is reduced as the diencephalon is enlarged, and this condition becomes progressively more evident from amphibians towards birds and mammals. In all mammals, however, the tectum retains its function of controlling the reflex turning of the head or eyes towards the source of external stimuli.

From the dorsal surface, mammalian tecta appear as a pair of hemispherical bulges in front of the cerebellum. Cell bodies in the tectum are organised into distinctive laminae arranged concentrically around the central grey matter, and each cellular band is separated by a layer of axons originating from

various parts of the brain. Axons stemming directly from the eyes terminate at the most superficial layer of the tectum, with those from the contralateral eye (crossed fibres) positioning slightly more superficially than those from the ipsilateral (uncrossed fibres). Two recent anatomical studies (Graybiel, *Brain Res.*, **96**, 1; 1975; Hubel *et al.*, *ibid.*, **25**) have revealed the intricate pattern by which terminals of optic axons from the two eyes are distributed on the surface of the tectum. The pattern varies systematically as fibres enwrap the tectum from the front to the back. On the posterior half of the tectum, for instance, clusters of uncrossed fibres form regularly spaced terminal arborisations giving the appearance of a chequerboard pattern. Crossed fibres, on the other hand, are distributed as a continuous sheet overlying the terminations of the ipsilateral fibres. The upper margin of this sheet is smooth and well defined, while the lower margin shows regularly spaced indentations interdigitating with the ipsilateral terminals.

Fibres from the visual cortex, which constitute the phylogenetically new secondary visual system, terminate at a slightly deeper level of the tectum, and there link up with the older direct retino-tectal system (Kuypers and Lawrence, *Brain Res.*, **4**, 151; 1967; Kawamura *et al.*, *J. comp. Neurol.*, **158**, 339; 1974). Although the mode of synaptic terminations of the direct and indirect visual fibres on tectal cells has not been clearly elucidated, it is known that a single tectal cell sometimes receives inputs from both sources (Sterling, *Vis. Res.*, **11**, 309, 1971; Hoffman, *J. Neurophysiol.*, **36**, 409, 1973)

In addition to the visual inputs, the

tectum receives projections from other sensory modalities, mainly the auditory, proprioceptive and somatosensory systems. These inputs originate from various parts of the brain, including the inferior colliculus (Powell and Hatton, *J. comp. Neurol.*, **136**, 183; 1969), auditory cortex (Garey *et al.*, *J. Neurol. Neurosurg. Psychiatry*, **31**, 135; 1968), spinal cord (Mehler, *Ann. N. Y. Acad. Sci.*, **167**, 424; 1969) and motor cortex (Sprague, *Anat. Rec.*, **145**, 288; 1963), and terminate at the deep layers of the tectum. Tectal neurones appear to form a synaptic chain, with superficially located cells synapsing onto successively deeper ones. At each level, extrinsic afferent fibres make further synapses with this intrinsic chain of neurones (Sprague, *Neurosci. Res. Prog. Bull.*, **13**, 204; 1975). The main output of the tectum is then channelled into the two motor nuclei (the Interstitial Nucleus of Ramon y Cajal and the Nucleus of Darkshevitich) and certain parts of the spinal cord that control eye and neck movements (Altman and Carpenter, *J. comp. Neurol.*, **116**, 157; 1961).

These anatomical descriptions accord with physiological studies. Cells in the superficial layers of the tectum have small visual receptive fields, responding optimally to a moving stimulus. Other cells, probably located slightly deeper than these simple cells, show several added complexities in their response pattern. For example, they show preference for a particular direction of movement, discharging briskly only when the visual stimulus is moving in a certain direction. After destruction of the visual cortex, this feature is seldom found, thus indicating that the cortical connections are responsible for this complexity (Wickelgren and Sterling, *J. Neurophysiol.*, **32**, 16; 1969; Rosenquist and Palmer, *Expl Neurol.*, **33**, 629; 1971; Berman and Cynader, *J. Physiol., Lond.*, **245**, 261; 1975). There is a marked increase in the size of receptive fields with increasing depth in the tectum, and cells in the deep layers respond to visual as well as somatosensory and auditory stimuli (Cynader and Berman, *J. Neurophysiol.*, **35**, 187; 1972).

Not only are the inputs to the tectum organised in laminae, they are also topographically ordered with respect to the external world, so that all the cells in a given column attend to the same part of the environment. Gordon, *Science*, **173**, 69; 1971; *J. Neurophysiol.*, **36**, 157; 1973) first showed that the optimum position of a sound source relative to the animal's head, for eliciting responses from a particular part of the tectum, corresponds with the positions of visual receptive fields of cells in the same part of the tectum. Moreover, if a

visual cell shows a particular preference for a given direction of movement, the deeper tectal cell responds best to a sound source moving in that direction. The columnar overlap of different sensory modalities has recently been re-examined (Dräger and Hubel, *Nature*, **253**, 203; 1975; *J. Neurophysiol.*, **38**, 690; 1975; Stein *et al.*, *Science*, **189**, 224; 1975). In both the cat and mouse, the map of the visual space is topographically coincident with the somatosensory representation of the animal's body. In the mouse, protruding whiskers cover the major part of the visual field. A tectal cell responding to gentle tapping of a whisker lies below a visual cell looking at that whisker. In those parts of the tectum involved in the visual field where no whiskers are in the way, somatosensory responses at deep levels are elicited from other parts of the body. For instance, inferior visual fields where the mouse may see its own paw are associated with tactile fields on the paw. The output of the tectum is then somehow coupled to the motor system such that the animal brings the source of stimuli into its central visual field. This has been demonstrated by directly stimulating different parts of the tectum electrically (Schiller and Stryker, *J. Neurophysiol.*, **35**, 915; 1972).

The hierarchical organisation of nerve cells, as found in the mammalian tectum, is the general principle on which all nervous systems are constructed. At each successive level, incoming messages are further processed, and the stimulus features to which a neurone will respond become more specified. Elucidating the rules whereby important features of the external world are extracted and recombined by the nervous system has been one of the fundamental problems in neurobiology. It is toward this aim that a group of neurobiologists are gradually building up the basic 'wiring diagram' of the tectum. What developmental mechanisms ensure the formation of such an exquisitely ordered structure are likely to remain unknown for a long time. □

Host selection by a parasitic mite

from F. E. G. Cox

MANY parasites make use of chemical signals to identify suitable hosts but in few cases have either the nature or the sequence of these signals been characterised. Arthropods usually exhibit a two-stage pattern of behaviour in which they first search for their hosts and then orientate themselves with respect to chemical stimuli emanating therefrom. Most studies on host finding have been based on insects such as



A hundred years ago

WE have received an address by Prof. R. H. Thurston, C.E., delivered to the graduating class of the Stevens Institute of Technology (U.S.). It is entitled "The Mechanical Engineer, his Preparation and his Work," and contains some excellent advice, useful not only to young engineers, but to all who have been trained to other mechanical professions. The Stevens Institute, though what we would call a technical college, affords a good general scientific training, with a fair admixture of literary culture, and the object of Prof. Thurston's address is to show that the more complete is the culture of an engineer, the greater is likely to be his professional success.

from *Nature*, **13**, 16; Nov. 4, 1875

mosquitoes that attack man (see for example Gillies and Wilkes, *Nature*, **252**, 388; 1974) but in this issue of *Nature* (page 788) Egan, Barth and Hanson describe how a mite that parasitises cockroaches locates and identifies its host. These authors used simple two-choice preferential tests and the attractant substances were applied to paper disks under controlled conditions. In all, the responses of 12,000 mites were examined. In itself, this study has little practical application in the immediate future, but it opens up possibilities of analysing the factors that cause mites to attack man and his domesticated animals and the development of methods of protection against these ectoparasites and the diseases they transmit.

The mite used was *Proctolaelaps nauphoetae* which is specific to and gregarious on the cockroach *Nauphoeta cinerea*. *P. nauphoetae* is first attracted to faecal materials and the sorts of things that occur in cockroach nests, such as pieces of limbs and rotting organic material. The identification and orientation towards such materials is likely to bring the mites to a cockroach nest, and it was confirmed that they can recognise nest "markers" because they preferentially seek out individuals that have been living in colonies, in contrast to those of the same species that have been living alone, and they are also attracted to non-host species that have been placed in host colonies.

Having come into the proximity of a cockroach colony, a second level of attraction enables the mites to identify cockroaches belonging to the same family as the host and to avoid others. The attractant consists of the expectorants which are spread over the body during grooming. The mite has then

to determine whether or not the cockroach is the appropriate host species. The actual substance that is used was isolated by placing paper disks in the colony and extracting the attractant from them. The authors tentatively identify this substance as a poly-amino sugar and call it "nauphoetamine". The identification of "nauphoetamine" by the mite is the final stage of the host-finding behaviour but it is important to note that chemical stimuli are involved in the first two stages: the recognition of the proximity of any kind of colonial cockroaches by faecal and detritus stimuli, and direct orientation to expectorants which brings the mite to a particular group of cockroaches which might or might not be the correct host species.

The fact that the mites show three separate levels of discrimination and that one at least of the attractants can be isolated means that it should be possible to attract mites away from potential hosts or towards pest species if biological control is the aim. Even if these studies do not lead to any further progress in the manipulation of mites they will be of general interest to parasitologists in several areas where host finding and identification are poorly understood and at present, in the case of warm blooded animals, often simply attributed to host temperature. □

Is submicrominiature small enough?

from Andrew Holmes-Siedle

SOLID-state science has made it possible to put all the circuits of a computer onto a piece of silicon no larger than a postage stamp (say 1 cm² in area). It is reasonable to ask how much further one should try to go. Should we now put the computer onto a pinhead or put a faster, more versatile computer with much more storage onto the same square centimetre? The idea has its attractions because we are never short of information to process (visual information flows in on us at more than 10 million bits per second, for example), and it saves energy if we cut down the amount of purified electronic material needed to make the circuits.

Two authoritative surveys on the ultimate limits to the density of electronic functions on a wafer of electronic material have appeared recently. One, by J. T. Wallmark (*Institute of Physics Conf. Ser.* No. 25, 133; 1975), starts from the practical necessities of integrated-circuit technology and, through original calculations, reaches quantitative conclusions. The other, by R. W. Keyes of IBM Research Centre (*Proc. IEEE*, **63**, 740; 1975) starts from some basic laws of device physics, collects results of many other workers and produces a set of conceptual tools rather

Comparison of physical limits in digital electronics

	Mathematical symbol	Units	Typical 1965 circuit	Typical 1975 circuit	Wallmark's ultimate circuit	Ultimate if pattern generation were only factor	Human brain
Number of transistors or other active elements	N		10	10^4	10^6	2.5×10^9	10^{10}
Linear dimension of chip	$\sqrt{A_c}$	μm	6.6×10^3 (0.66 cm)	10^4 (1 cm)	6.6×10^3 (0.66 cm)	10^4 (1 cm)	—
Area of chip	A_c	μm^2	4×10^7	10^8	4×10^7	10^8	—
Area per active element with allowance for interconnections	A_c/N	μm^2	4×10^6	10^4	4×10^3	4×10^{-2}	—
Side of active element (e.g. whole transistors)	$\sqrt{A_c/N}$	μm	2×10^3	10^2	20	2×10^{-1}	4
Linewidth, d , required to fabricate element	$10^{-1}\sqrt{A_c/N}$	μm	200	10	2	2×10^{-2}	—
Minimum edge uncertainty, ΔL , for above linewidth	$5 \times 10^{-4}\sqrt{A_c/N}$	μm	1	5×10^{-2}	10^{-2}	10^{-4}	—
Wavelength of light required	$2\Delta L$	μm	2	0.1	2×10^{-2}	—	—
Energy of electron beam required	$2h^2/m\Delta L^2$	eV	$\ll 1$	< 1	~ 0.1	100	—

than quantitative conclusions. The two are thus complementary, and provide a good foundation for the statement that we cannot much longer continue the trend by which the density of devices per unit area is doubled annually. Physical laws will in fact soon prevent us. The limiting processes include the propagation of light, thermal conduction, electromigration and dielectric breakdown. The first three columns of the table, derived loosely from Wallmark's and Keyes' formulae, show where the downward trend in size must stop. While, in the past ten years, the area of an active, multielement structure such as a transistor has gone down from about 1 mm^2 to 0.01 mm^2 , giving a minimum element width, d , of $10 \mu\text{m}$, Wallmark gives good arguments as to why it can never go lower than $2 \mu\text{m}$. One major problem, very difficult to circumvent, is that the local concentration of doping impurity atoms fluctuates noticeably on this scale so that small elements such as resistors, intended to be of the same value, will in fact fluctuate in value. Wallmark is alone in seeing this as the main physical barrier, but both authors find that active devices, such as transistors can only pass a certain current density before breakdown phenomena occur, and the size limit for this effect again occurs when d is a few micrometres.

It is worth noting that the technology for making linewidths much smaller than $2 \mu\text{m}$ is well in hand. While conventional ultraviolet light cannot be used near Wallmark's limit (see table), the wavelengths of low-energy electron beams are such that much smaller patterns could be made with ease. I would estimate that, if pattern generating were the only limit, one could fit more than 10^9 transistors on a chip of area 1 cm^2 (see fourth column of table), but electrical breakdown, heating and electromigration effects would attend the high current densities and electric fields which these much smaller elements would experience. The reason why fields increase as size decreases stems from the existence of voltage noise. Random voltage fluctuations of value kT (0.025 eV at 300 K) occur across

junctions and the applied voltages must swamp these. Thus, voltage cannot be scaled down with element size. Keyes further insists that, with all digital logic devices, the mode of operation must be such that large non-linearities in the I-V characteristics are involved. This requires operating voltages of 10 – 100 times kT . In non-digital systems, however, smaller energy transfers can possibly be used to process information. Keyes gives an interesting study of the bare essentials of a logic operation, namely two potential wells with a small barrier between them, the barrier being of the order of kT . In the ideal case, the energy dissipated in one operation of this system is of the order 10^{-21} J at 300 K and this is the quantum limit. By contrast, low-power logic uses about 10^{-10} J per operation. The only known information-processing system which approaches this efficiency is a natural one—the passage of information during nucleic acid transcription, which involves about 10^{-19} J per operation. The advantage which nature has here is that speed of processing is of little importance to cell growth. The human brain, requiring greater speed of operation, operates at about the same energy per operation as low-power electronic logic.

A difference in conclusions on minimum size, as reached by Keyes, Wallmark and another earlier study (Honeisen and Mead, *Solid St. Electron.*, **15**, 819, 891; 1972) lies mainly in their ideas of which elements of the circuit will in future be the controlling ones. For this, Keyes chooses the interconnections between devices, in which the speed of light will limit speed of transport and high current densities can produce heating and electromigration (minimum linewidth, $10^{-1} \mu\text{m}$). Honeisen and Mead see dielectric breakdown as an earlier barrier (minimum width, $1 \mu\text{m}$) while, as mentioned, Wallmark takes the broader view and considers that the statistics of pattern generation (not fully considered by the others) and doping fluctuations will raise the limiting width well above $1 \mu\text{m}$. Agreement on the minimum power dissipated for a given speed of

operation is fairly good. At room temperature, voltage fluctuations and the capacitances of components will not allow speeds of operation faster than a few tenths of a nanosecond or power levels below a nanowatt per element. In special cases, cooling to cryogenic temperatures can lead to lower power levels, although commonsense suggests that almost all the uses for the most minute logic devices will tolerate neither the inconvenience nor the power drain involved in continuous cooling. It seems that, unless we can develop some very unusual electronic materials, the only way to encompass extremely small size in computers is to accept a slower-than-normal rate of operation. Furthermore, we might as well accept that we will never get the equivalent of a videotape recorder or Atlas computer which will fit comfortably in the pocket.

Hormonal regulation of hormone receptors

from J. R. Tata

THE central issue in research on hormone action is a better understanding of hormone-receptor interaction. Until recently, kinetic analyses of hormone receptor function have been based on the assumption that the number of receptor sites per target cell is constant, or that its fluctuations are of little physiological consequence, so that what really matters is the number of hormone molecules that are available for interaction with receptor sites. Reports are, however, now trickling in to suggest that the number of receptors per cell can vary sufficiently to influence the response of the target cell to the hormone. What renders this idea particularly intriguing is the possibility that a hormone can modulate the cellular level of its own receptor or that of another hormone.

Hinkle and Tashjian, Jr. (*Biochemistry*, **14**, 3845; 1975) have recently examined the effect of thyrotropin-releasing hormone (TRH), a hypothalamic tripeptide, on the number of

receptors for this hormone in a clonal strain of rat pituitary tumour cells (GH₃ cells). These cells do not make thyrotropin (TSH) but TRH does stimulate the synthesis and release of the hormone prolactin and inhibits the formation of growth hormone by these cells. Previously it had been shown that TRH bound firmly to GH₃ cells, presumably to receptors on plasma membranes of the type found in normal pituitaries (Vale *et al.*, *Endocrinology*, **93**, 26; 1973; Gourdji, *et al.*, *Expl Cell Res.*, **82**, 39; 1973) and that the binding preceded the action of the hormone. According to Hinkle and Tashjian, incubation of GH₃ cells for 2–3 days with TRH caused a reduction in the number of binding sites for TRH to a third of the normal level. Receptor molecules seem to be long-lived and their loss itself requires active protein synthesis since cycloheximide protects the cells against receptor depletion. A consequence of such a hormone-induced loss in receptor capacity would be that it would provide a target cell with the means to regulate its sensitivity to the hormone.

Is the above example of a hormone regulating the number of its own receptor molecules a peculiarity of abnormal tumour cells or of culture conditions, or does it reflect a universal physiological homeostatic mechanism for hormones? After all, GH₃ cells do not synthesise TSH whereas the action of TRH on normal pituitary cells is to control their TSH output. In another pituitary tumour cell line, GH₁ cells, whose growth is influenced by thyroid hormone, Samuels and Tsai reported that tri-iodothyronine (T₃) increased the number of T₃ receptors in the nucleus (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3488; 1973). But, Spindler *et al.* (*J. biol. Chem.*, **250**, 4113; 1975) have failed to observe a similar phenomenon for the number of T₃ receptors in normal rat liver nuclei. It may, therefore, be premature to generalise about the self-regulation of hormone receptors. Perhaps one needs to examine those systems where sensitivity of a tissue to a hormone changes very rapidly, as during ontogenesis or during certain hormone-dependent cyclical physiological situations. It may well be that the basis of the "positive feedback" phenomenon during amphibian metamorphosis may be due to marked changes in the number of T₃ receptors in the tadpole hypothalamus. Similarly, oestrogenic hormones regulating the level of their own receptors may underlie the cyclical variations in the hormonal sensitivity of the uterus during the oestrus cycle. In this context, an interesting example of induction of oestrogen receptor by oestradiol was reported by Gschwendt and Kittstein (*Biochim. biophys. Acta*, **361**, 84;

Eruption anticipated

from Peter J. Smith

ON July 5–6 this year the Hawaiian shield volcano, Mauna Loa, erupted for the first time in 25 years. The renewed activity was not entirely unexpected, however, for only a few weeks before the eruption scientists at the US Geological Survey's Hawaiian Volcano Observatory had prepared a report, based on seismic and geodetic data, suggesting that Mauna Loa "may be reawakening". As it turned out, the report was overtaken in press by the event, but now that it has been published (Koyanagi *et al.*, *Geophys. Res. Lett.*, **2**, 405; 1975) it is possible to see the evidence upon which the prediction was based.

Since Mauna Loa last erupted in 1950, seismic activity in its vicinity has been low but not completely absent. Records covering the past 13 years show that there were several bursts of activity which reached small peaks in 1962, 1967 and 1970, each larger than the one before. But in April 1974 the frequency of small summit earthquakes increased con-

spicuously, reaching hundreds to thousands a day for a week in mid-August, more than a week in mid-December and for many days between February and June 1975. Throughout the whole period 1962–1975 magnitudes ranged up to 4.6 but most of the events with magnitudes greater than 3 occurred during 1974–75.

During the latter period the shocks generally became not only larger but also shallower than before. Most of the foci were at depths of less than 15 km, although a few reached 50 km. Moreover, there was an earthquake-free zone at depths of 15–30 km beneath the summit crater, believed to indicate a magma storage region. The epicentres defined a broad seismic zone around the summit where dilation was evidently also occurring. Thus geodimeter measurements showed that a 2.5 km line across the summit caldera expanded by 10 cm between August 1974 and June 1975.

Taken together, these data strongly indicated a resumption of volcanic activity, although they were far from sufficient to enable the time of any eruption to be predicted.

1974). These workers demonstrated that injection of oestradiol to male chicken leads to the induction of high affinity nuclear binding sites (K_D of about 10^{-9} M) in the liver under conditions in which the hormone induces the synthesis of egg-yolk proteins in this tissue.

Perhaps a more common situation in which hormonal modulation of hormone receptor level may have physiological relevance is that in which one hormone controls the level of receptors for another hormone. Several complex, multi-hormone systems are known whereby one hormone conditions a tissue or a group of cells in its response to another. Two examples of such hormonal interplay are lactation, in which steroid hormones induce the mammary epithelial cells to become sensitive to the lactogenic hormone, prolactin (see Topper, *Recent Prog. in Hormone Res.*, **26**, 287; 1970) and the sensitivity of the chick oviduct to progesterone being dependent on previous stimulation by oestradiol (see O'Malley *et al.*, *Recent Progr. in Hormone Res.*, **25**, 105; 1969). It is therefore of some interest that oestrogen induces a cytoplasmic 7S receptor for progesterone both in the chick oviduct (Toft and O'Malley, *Endocrinology*, **90**, 1041; 1972) as well as in the hamster uterus (Reel and Shih, *Acta Endocr.*, **90**, 344; 1975). With interest in the above phenomena increasing rapidly, it is safe to predict that many "hormone receptorology" papers will be dealing with the regulation of hormone

receptor levels. Whatever the outcome of such efforts, the important issue will still be the perennial question of how does one equate hormone binding with the site, or sites, of relevant physiological actions of a given hormone.

A most Friendly meeting

from Natalie Teich and Paul Harrison

The Friend Cell Workshop was held at Schloss Reisenburg, West Germany, on August 28–September 1.

THE workshop established the relevance of the Friend virus–Friend cell system as a model for erythropoietic differentiation, for multiple host gene control of erythroblastic disease and for studies of the relationship between cell transformation and abnormal differentiation.

The cell biology of the Friend cell system was a major topic. Friend virus (FV) infection of susceptible mice results in enhanced erythroblastosis and polycythemia or anaemia (the original virus strain of Dr C. Friend) due to massive cell death in the early erythroblast compartment (C. Jasmin, Hôpital Paul Brose, Villejuif). It now seems established that the target cell for FV is a committed erythroid stem cell (CFU-E) (P. Tambourin, Radium Institute, Paris) Axelrad's group (Uni-

versity of Toronto) have isolated a strain of virus able to transform normal CFU-Es *in vitro* as judged by their ability to differentiate without erythropoietin. This may be analogous to the *in vitro* transformation of avian bone marrow cells by avian erythroblastosis virus (AEV) (T. Graf, Max-Planck-Institut, Tübingen).

A diversity of studies have evolved from the isolation of Friend erythroleukaemic cells (FLC) *in vitro* (C. Friend, Mount Sinai School of Medicine, New York). These cells are CFU-Es arrested at the proerythroblast stage, which after treatment with dimethylsulphoxide (DMSO) synthesise haemoglobin and differentiate to orthochromatic erythroblasts and possibly further (Y. Ikawa, Cancer Research Foundation, Tokyo). Other erythroid changes follow: spectrin formation and loss of H-2 surface antigen (H. Eisen, University of Geneva) and accumulation of carbonic anhydrase (F. Ruddle, Yale University). New compounds, such as highly polar molecules and fatty acids (R. Rifkind and P. Marks, Columbia University, New York) also act as inducers; haem also can induce globin synthesis and may act synergistically with DMSO (J. Ross, McArdle Laboratory, Wisconsin; A. Beaudet, Baylor College, Houston). This induction of erythroid differentiation in FLC contrasts with the situation in AEV-transformed avian erythroid cells which do not differentiate or produce haemoglobin in response to classical inducers of FLC (Graf), although they accumulate globin RNA in the nucleus (A. Therwath, University of Paris).

Little is known concerning the mechanisms of action of the various inducing agents. Membrane function may be important, however. A. Bernstein (Ontario Cancer Institute, Toronto) found that local anaesthetics which increase membrane fluidity block haemoglobin production. This is consistent with other membrane changes including rapid immunological "capping" (N. Furusawa, Osaka City University), increased agglutination with plant lectins (Eisen), decreased permeability to small molecules (S. Dube, California Institute of Technology), increased anisotropy (D. Jovin, Max-Planck-Institut, Göttingen) and changes in cyclic nucleotides (R. Kram and P. Malpoix, University of Brussels).

Regarding the cell cycle dependence of the induction process, haemoglobin is not detectable until cells have been exposed to DMSO for about two cell cycles (J. Papaconstantinou, Oak Ridge National Laboratory; Marks and Rifkind). However, J. Pragnell (Beatson Institute, Glasgow) and W. Ostertag (Max-Planck-Institut, Göttingen) reported that one FLC line shows

a 5-fold increase in globin mRNA within the first cell doubling.

Several laboratories have attempted to elucidate the mechanisms regulating globin gene expression in FLC, exploiting DNA complementary to globin mRNA to measure synthesis and accumulation of globin-specific RNA. H. Aviv (Weizmann Institute, Rehovoth) stressed that relative rates of degradation of globin and total mRNAs are important in understanding how globin mRNAs predominate in the reticulocyte or differentiated FLC. Unfortunately even the sensitive techniques used are insufficient to permit similar analyses at the nuclear level to clarify the role of transcriptional and post-transcriptional regulation mechanisms. The fact that the base-sequence complexity of poly(A)-containing nuclear RNA is considerably greater than that of polysomal poly(A)-containing RNA indicates post-transcriptional control of gene activity (M. Birnie and J. Paul, Beatson Institute, Glasgow).

Another approach to regulatory mechanisms was described by P. Harrison and D. Conkie (Beatson Institute) utilising non-inducible FLC variants. In some, non-inducibility of globin synthesis is due to a dominant

defect at the nuclear level (either in globin gene transcription or an unstable primary RNA transcript); whereas in another, treatment with haem enhances globin and globin mRNA accumulation. Interestingly, in a lymphoma \times FLC hybrid, globin mRNA accumulates, but globin chains do not; nor does erythroid maturation occur unless the hybrid cells are treated with haem in addition to DMSO. A regulatory role for haem in differentiation of FLC is also implied by the report of G. Kramer and B. Hardesty (University of Austin, Texas) that there are two inhibitors of translation in Friend cells, one of which is present in DMSO-treated cells only and bears close similarity to the classical haemin-controlled repressor present in reticulocytes.

Others are also using cell fusion to analyse the regulation of differentiation. B. Alter (Childrens Hospital, Boston) showed that some human globins were synthesised in heterokaryons between tetraploid FLC and diploid human amniotic fibroblasts but not in hybrids with diploid FLC. A. Skoultschi (Albert Einstein, New York) observed that hybrids between FLC and mouse hepatoma cells continue to synthesise albumin and transferrin but cannot be induced to synthesise haemoglobin or globin mRNAs. The potential of somatic cell genetics for genetic mapping was illustrated by A. Deisseroth (National Institutes of Health) by exploiting stable mouse-human hybrid clones to correlate loss of human globin genes with loss of specific chromosomes. It should only be a matter of months before the location of the α and β genes are known with certainty.

As regards the virus-host interactions, it is clear that at least three genes of the mouse determine the course of the erythroblastic disease induced by FV. The *Fv-1* locus controls the ability of the helper murine leukaemia virus (MLV) component to initiate productive infection. A second gene, *Fv-2*, controls the animal's response to the spleen focus-forming element (SFFV) of FV; in a resistant mouse, MLV and SFFV replicate and although morphologically distinct spleen foci do not appear, splenomegaly occurs later (5 weeks) (T. Odaka, Institute of Medical Science, Tokyo). K. Blank (Albert Einstein, New York) showed the restrictive effect of each locus not only on the spleen focus formation but also on the ability of the animal to limit growth of the tumour colony-forming cells. A third locus, the major histocompatibility locus (*H-2*), seems to affect not the initial infection by FV, but rather the subsequent ability to support chronic infection (H. Freedman, Albert Einstein,

Chemical and biological reactions

IN my recent comments (*Nature*, 256, 693; 1975) on dynamics of chemical and biological reactions I mentioned work by Uzgis and Golibersuch (*Phys. Rev. Lett.*, 32, 37; 1974) attributing a decay term independent of scattering angle in scattered-light intensity fluctuations from haemoglobin solutions to the haemoglobin association-dissociation reaction. A paper by Haas, Mustacich, Smith and Ware (*Biochem. biophys. Res. Commun.*, 59, 174; 1974) which disputes these findings has now come to my attention. These authors attribute the "anomalous" positive intercepts in the plot of linewidth *versus* the square of the scattering vector reported by other authors to the coincidence of the Helium-Neon wavelength and the 630 nm absorption of methaemoglobin. I am bringing these findings to the attention of your readers in view of the importance of establishing whether reaction rate of fast chemical and biological reaction can be derived from time autocorrelation functions.

I would also like to state that the fluorescence of ethidium bromide is strongly enhanced, and not quenched (as mentioned in my comments) upon binding to DNA.

H. EISENBERG

New York). G. Hunsmann (Tumor Immunology, Freiburg) reported that FLC express viral surface antigens gp71 and p12; further, immunisation either with gp71 or antiserum to gp71 will protect animals from FV disease.

Ostertag showed that treatment of FLC with BUdR and DMSO often leads to the induction of endogenous viral genomes, thus complicating the host range patterns of the Friend virus complex. Pragnell reported that sequences present in Friend virus released from DMSO-treated FLC are integrated 5-10 times in Friend cell DNA; whereas about half of these sequences (SFFV?) are absent in mouse embryo DNA. Ostertag and P. Swetley (Boehringer Institute, Vienna), as well as Ruddle, have shown that interferon treatment will effectively block virus replication without inhibiting haemoglobin synthesis. Other agents such as azidothymidine act similarly. Ostertag does, however, find a correlation between induction of differentiation and increase in intracisternal A-type particles.

N. Kluge (Max-Planck-Institut, Göttingen) and Sugiyama have established several rat erythroleukaemia lines by treating animals with dimethyl benzanthracene; these lines are induced for haemoglobin synthesis with DMSO and for virus production with BUdR. B. Weimann's (Immunology Institute, Basel) human polycythemia vera lines are not responsive to erythropoietin, but A-type particles are seen after treatment with azidothymidine. Thus, the Friend cell phenomenon is not restricted to the mouse. □

Man-made earthquakes

from E. Nyland and D. I. Gough

The first International Symposium on Induced Seismicity (ISIS) was held in Banff, Alberta, Canada, from September 15 to 19. ISIS was organised as a consequence of the Conference on Seismic Effects of Reservoir Impounding held in London in 1973, and both were instigated and supported by a working group of UNESCO. ISIS was additionally supported by the Government of Canada and was organised from the Universities of Alberta and British Columbia. The Proceedings of ISIS will appear late in 1976 in *Engineering Geology*.

AMONG engineering activities which have inadvertently produced earthquakes are the building of dams which

form large reservoirs, the injection of water into crustal rocks for waste disposal or oil extraction, and deep mining. All three topics were actively discussed, although the bulk of the papers were concerned with reservoir-triggered earthquakes.

D. W. Simpson (Lamont Doherty Geological Observatory, New York) led the papers on this topic with a comprehensive review of large reservoirs, only a small fraction of which are known to have triggered seismic activity. Among the contributed papers was one by O. V. Soboleva and colleagues (Academy of Science, Padzhik SSR), presented by Simpson who is visiting Dushanbe, USSR on a US/USSR collaborative project on detailed studies of seismicity induced by Nurek Reservoir, formed by the world's highest earth-fill dam. Epicentres migrated to the southwest end of the lake from a distance of 8 km over the years 1971-74. Focal mechanisms are complex. Six seismologists and engineers from the People's Republic of China reported on admirably thorough studies of the intense seismic activity induced by the Hsinfengkiang reservoir, where some quarter million seismic events have been detected since the lake began to fill. At least four mechanisms have been involved: initial normal faulting may have been load-induced and facilitated entry of water to deeper foci for larger subsequent strike-slip events. Another major field study, this time of the Tarbela dam in a region of very high natural seismicity in the Pakistani Himalayan foothills, was reported by Klaus Jacob (Lamont Doherty). Current discussions of earthquake control by fluid injection lend interest to a paper by C. G. Bufe (US Geological Survey) reporting a gap in seismicity on the Calaveras Fault in California: the gap is near the small Anderson Reservoir and elsewhere there are many events along the fault.

A few generalisations can be stated. Initial stress is clearly a principal factor in the triggering of earthquakes by reservoirs, but techniques for its measurement remain costly and of doubtful generality. They are almost never applied except near the dam itself. Fluid pressure is probably the main triggering mechanism, although incremental solid stress can serve where normal faults are critically prestressed. In the largest earthquakes triggered by reservoirs (Kremasta, Kariba, Koyna, Hsinfengkiang) magnitudes of mainshocks do not exceed 6.3, an empirical observation which may be related to the rock volume affected by very large reservoirs.

Carl K. R. Ling (Cooperatives Institute Research in Environmental Sciences, Boulder, Colorado) gave a review of theory of induced seismicity, which

finds its basis in the principle of effective stress in a porous water-filled medium and Coulomb-Mohr shear failure criteria. The extensions of these simple notions to real permeable formations has become immensely complicated. The following session showed encouraging moves toward an attack upon calculation of stress fields in porous, fractured media. Tidal triggering seems possible under at least one reservoir.

A session on mechanical and hydraulic properties of rocks related to induced seismicity was led by P. A. Witherspoon (University of California, Berkeley) with a survey of a vast body of results from his laboratory. Contributed papers considered properties of fractures, friction and sliding in rocks.

Seismicity associated with deep mining differs from induced seismicity of other types in that the excavation can itself produce stress-differences sufficient for failure, so that the effect need not, in all cases, be a triggering: initial stress may thus be lithostatic. Another special feature of this class of event is that the focal region can be visited and studied before and after the shock. A review of many years' study in Witwatersrand gold mines was given by N. G. W. Cook (Chamber Mines, South Africa). A feature of work in the central Witwatersrand is the extreme dryness of the rock there. Contributed papers included discussions of seisms produced by longwall coal mining in the United States and of seismic efficiency of mine tremors in the Witwatersrand. An earthquake sequence triggered by unloading of rock from a quarry in the Hudson valley is really explained in terms of thrust-faulting mechanisms and reduction of the vertical (least principal) stress.

A swarm of earthquakes about 10 km from a reservoir several years old, near Oroville, California, is following a mainshock on August 1, 1975. The timing was excellent in relation to ISIS, and an evening session was organised by Lloyd Cluff (Woodward-Clyde Consultants, Oakland, California). Studies by his consultant group, by the US Geological Survey and by the University of Nevada contributed to a fascinating case-history but leave unanswered the question whether the reservoir induced the earthquakes, which occurred on an active fault.

J. H. Healy (US Geological Survey) led a session on seismicity associated with fluid injection with a lucid review of results from the US Geological Survey investigations into the Denver earthquakes and the Rangely Oilfield earthquakes, both in Colorado. His was perhaps the most up-to-date review, being completed on the morning of the

session. In contributed papers there were discussions of seismicity related to hydraulic mining in New York state and seismicity associated with a complex of oil, water and gas at different levels under Lacq, France.

In seismicity related to fluid injection, fluid pressure is clearly the trigger and as with reservoirs, the pre-existing fractures and stresses are all-important and in general only become known if and when earthquakes occur. Pulling the trigger is not the best way to find out whether a gun is loaded; measurement of initial stress near future reservoirs and near oilfields where high-pressure injection is proposed could be a useful exercise for government agencies.

A session on instrumentation for observation of induced seismicity was led by a review by E. R. Kanasevich (University of Alberta), who made a strong case for introduction of digital recording systems employing miniaturised solid-state electronics and dedicated minicomputers. This triggered a brisk discussion in which the merits of many simple instruments were urged by others, particularly in adverse environments far from shop support.

The six review papers and over fifty contributed papers provoked lively and absorbing discussions and it is a matter of regret that more seismologists, and especially more engineers, did not attend. For those who did, the week seems to have been well spent. □

Growth hormone at Milan

from Mike Wallis

The Third International Symposium on Growth Hormone and Related Peptides was held in Milan on September 17–20. Abstracts have been published (*Ricerca Scientifica ed Educazione Permanente*, 2, Suppl. 1) and the invited lectures will be published in full by *Excerpta Medica* in 1976.

PROTEINS come in families, and this conference covered all the members of the pituitary growth hormone family, including prolactin and placental lactogen. The ground covered was thus broad (over 160 papers) and attention here will be concentrated on growth hormone (GH).

A topic which raised much discussion was the possibility that pituitary GH itself is in fact a prohormone. S. Ellis and R. E. Grindeland (NASA-Ames Research Centre, California) described studies on the nature of GH in human

plasma, and concluded that the circulating form has enhanced biological activity and reduced immunological activity compared with GH extracted from pituitaries; purification of GH from plasma is underway. U. J. Lewis *et al.* (Scripps Clinic, La Jolla) have detected various forms of GH (apparently produced by limited proteolytic cleavage) with enhanced biological activities; these are obviously candidates for the 'activated' form which appears to circulate in the blood. M. A. Vodian and C. S. Nicoll (University of California) found that bioassay and immunoassay gave widely differing results for circulating GH in the rat (and for GH secreted by pituitaries incubated *in vitro*) although the two assays agree well when used to measure the GH content in the pituitary of resting, untreated rats.

The suggestion that GH is modified *in vivo* accords well with observations that active fragments can be produced by limited proteolysis of the hormone *in vitro*. C. H. Li and T. A. Bewley (University of California) and J. L. Kostyo *et al.* (Emory University) described the characterisation of peptides from plasmin digests of GH which retain biological activity in several different assay systems. L. Graf *et al.* (Research Institute for Pharmaceutical Chemistry, Budapest) described a similar approach using thrombin. Active fragments have also been synthesised chemically, as described by F. Chillemi *et al.* (University of Milan). How these studies on active fragments produced *in vitro* can be related to those produced *in vivo* is not yet clear. One attempt to make the link has been made by J. Bornstein (Monash University) who has isolated (from pituitaries) small peptides apparently derived from growth hormone. These have been synthesised chemically, and have been shown to be active in a range of pharmacological and biochemical test systems, but how they relate to the other work on GH fragments, or to the major biological actions of GH, remains uncertain.

Biosynthetic precursors of GH have been suggested on several occasions, and a paper by F. C. Bancroft (Columbia University) provided an elegant demonstration of the probable existence of such a precursor. Messenger RNA (from a line of pituitary tumour cells) was prepared and translated in a cell-free system from wheat germ. A major product possessed immunological and chemical resemblance to GH, but was 20% larger than the normal hormone. It appears that the wheat germ system (unlike mammalian cell-free systems) lacks the enzymes which process the GH-precursor (pro-GH or perhaps pre-GH); a similar situation has been demonstrated for several other

polypeptide hormones. The existence of a precursor was also proposed by M. Wallis (University of Sussex) to explain the N-terminal heterogeneity seen in bovine GH.

A major topic of discussion with regard to the biological actions of GH was the role of somatomedins (GH-dependent peptides found in serum). It is not yet clear whether these mediate all the actions of GH, including growth promotion, or whether GH possesses some direct metabolic activity in addition to its role in promoting somatomedin production. Exciting progress in the somatomedin field was described, though it is unfortunate that some work is shrouded in secrecy as a result of the interest of the pharmaceutical industry. At least three somatomedins have now been recognised, distinguished by their relative potencies in different assay systems. Isolation and characterisation of somatomedins A and B was described by L. Frykland and H. Sievertsson (AB KABI, Stockholm). They are moderate-sized peptides, structurally quite different, and neither is a fragment of GH. K. Hall and K. Takano (Karolinska Hospital, Stockholm) described their radio-receptor assays and immunoassays for somatomedins A and B. Levels in normal human serum are quite high (about $2 \mu\text{g ml}^{-1}$ for somatomedin A, $10 \mu\text{g ml}^{-1}$ for somatomedin B). As expected, levels rise in acromegaly and fall in hypopituitary dwarfism. The somatomedins are not completely absent in the latter, however, so these factors are perhaps only partially GH dependent. L. E. Underwood *et al.* (University of North Carolina) described work on somatomedin C (a moderate-sized, basic peptide) and showed that receptors for this occur in most tissues.

Lactogenic hormones can be mentioned only briefly here. R. E. Fellows (Duke University) has characterised ruminant placental lactogens and preliminary structural studies suggest that, as has been predicted, these differ substantially from human placental lactogen.

The idea of holding a conference in which all members of the GH-prolactin family of proteins were considered was an excellent one. Although the growth hormones and lactogenic hormones are clearly related structurally, and must have had a common evolutionary origin, little resemblance has been detected at the biological level. It seems likely that structural resemblances are paralleled by resemblances in biochemical mode of action but these have not yet been clearly established. Perhaps this will be one of the themes of the 4th International Symposium on GH in four years' time. □

review article

Lateral heterogeneity and mantle dynamics

Thomas H. Jordan*

Recent work on the nature of the Earth's lateral heterogeneities yields two conclusions with implications for mantle dynamics: seismic velocity differences between continents and oceans extend to depths exceeding 400 km, and strong lateral velocity gradients at depths greater than 800 km characterise the mantle beneath many subduction zones. These results suggest that more than just the crust and upper mantle are involved in whatever form of convection is responsible for plate motions.

THE study of seismic wave velocities provides the most detailed information about the structure of the Earth's interior and its internal variation of material properties. Until quite recently, it was the consensus among seismologists that below the uppermost mantle the velocity distributions show little geographic variation. With the collection of better data and the application of high-resolution techniques during the past decade, however, it has become clear that this view cannot be maintained. Evidence is rapidly accumulating which indicates the existence of significant lateral heterogeneity throughout the Earth's mantle, even at great depths. Here I attempt to synthesise some of the evidence. Particular attention will be focused on two results which constrain the nature of mantle dynamics.

Continent-ocean heterogeneity

The extreme geographical variations in upper mantle velocities are evidenced by the large-magnitude differences in station anomalies¹ and in regional surface-wave dispersion curves². The available seismic data suggest that the large scale lateral heterogeneity of the upper mantle is largely a consequence of the structural differences between continents and oceans.

Because of the dearth of seismic recording stations in oceanic regions, it is difficult to probe oceanic upper mantle structure using conventional body-wave methods, and most of our information on oceanic structure has come from the study of surface-wave dispersion. Dispersion studies using Love and Rayleigh waves reveal that continental and oceanic surface-wave phase velocities differ out to periods exceeding 300 s (refs 3–6), and from the analysis of these data a reasonably clear picture of the structural differences in the upper 400 km of the mantle has emerged: below a depth of 50 km or so the upper mantle beneath oceans is characterised by a well developed shear-wave low velocity zone (with contrasts as high as 0.5 km s^{-1}), whereas beneath stable continental shields this low velocity zone is weakly

expressed and in some regions it may be nonexistent. Since the shear-wave low velocity zone is associated with a zone of partial melting⁷, the simplest explanation of this difference seems to be a depression of the isotherms beneath the continents, an interpretation that is in accord with modern thermal models.

But this raises a critical question with implications for mantle dynamics: what is the maximum depth to which these continent-ocean differences persist? The popular consensus is that continental plates are on the order of 200 km thick (or less) and that below this depth the sub-continental and sub-oceanic mantles are similar in composition and state^{8,9}. If this model of thin lithospheric plates is correct, then, below a depth of 200 km or so, we should observe very little structural contrast between continents and oceans.

It was first thought that the observed differences in the surface-wave dispersion data required continent-ocean heterogeneity as deep as 500 km, and this evidence was used to argue against the continental drift hypothesis¹⁰. Dziewonski¹¹ has demonstrated, however, that the phase velocity differences may be explained by models with the significant velocity contrasts confined above 200 km depth, which is consistent with the thin-plate concept of having continent-ocean differences concentrated in and above the low-velocity zone.

Although this conclusion is compatible with current concepts, it requires substantial modification^{12,13,18}. Evidence for continent-ocean heterogeneity below the low velocity zone has come from the inversion of free oscillation data and from recent work with shear-wave travel times.

When travel times through the upper mantle beneath the continents are compared with those predicted by the free oscillation models, surprising differences are found: travel times for the average Earth are considerably larger than expected. As shown in Fig. 1, the one-way vertical shear-wave travel times through the upper 700 km of the Earth computed from the free oscillation models (for example UTD124A or B1) are 2–3 s greater than those obtained by the direct measurement of travel times at continental-based seismic recording stations (for example the Jeffreys-Bullen Tables or SLUTD1). Jordan and Anderson¹⁵ have postulated that this baseline difference is due to continent-ocean heterogeneity. Because the eigenperiod data yield estimates of the spherically averaged velocities and because the Earth's surface is two-thirds ocean, this hypothesis requires

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that the average one-way travel-time difference between continents and oceans be 3–5 s for shear waves. This compares with a difference of only 1–2 s predicted by regionalised Earth models with heterogeneity confined above 400 km (for example models O1 and S1 in Fig. 1, and ref. 17). The implication is that continent–ocean differences extend below this depth.

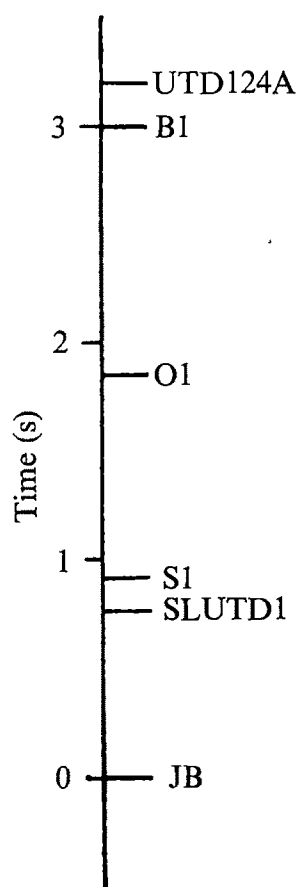


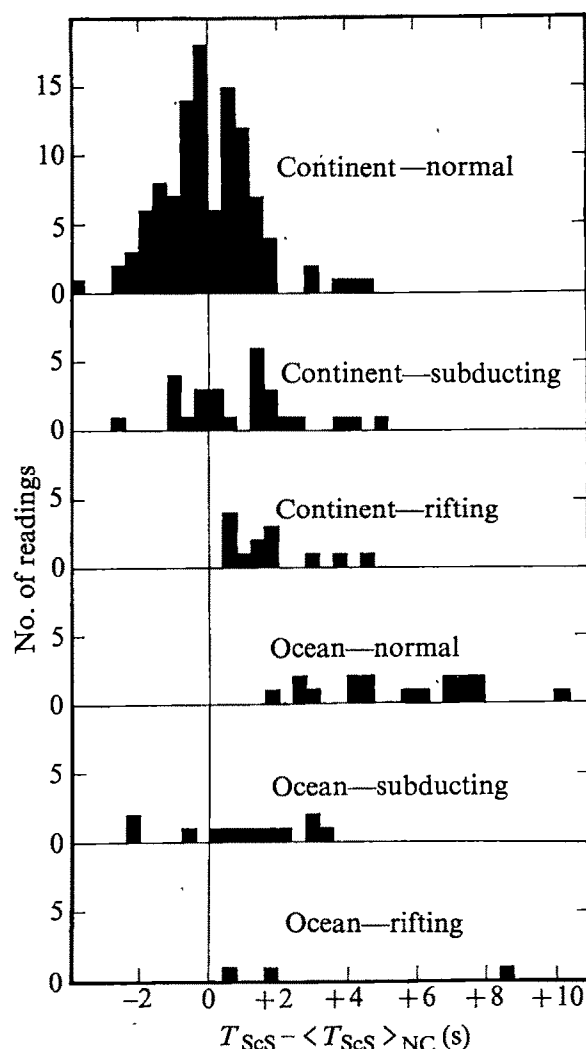
Fig. 1 Differences in one-way vertical shear-wave travel time from a depth of 700 km to the surface predicted by various velocity models. The JB time (143.6 s, ref. 16) has been subtracted to form residuals. Models UTD124A (ref. 14) and B1 (ref. 15) are gross Earth models which satisfy the free oscillation data; model SLUTD1 (ref. 29) is a shear-wave velocity model derived from continental based travel times; and models S1 and O1 (ref. 11) are regionalised models which satisfy the pure-path surface-wave dispersion data for shields and oceans, respectively.

Direct measurements of vertical travel-time differences for shear waves propagating through the continental and oceanic upper mantles have been made using ScS and multiple ScS phases. These times confirm the existence of a 3–5-s difference inferred from the free oscillation work. Figure 2 summarises the ScS travel-time differences from deep focus events observed by Sipkin and Jordan¹⁸. ScS was used because its travel-time curve is not sensitive to radial variations in velocity and because it seems to be less affected by lower mantle heterogeneity than the direct S phase (see below). In this study the seismic stations were crudely classified into six categories, each representing a particular tectonic setting. The four stations placed into the "normal oceanic" category were Kipapa (Oahu), Afiamalu (Samoa), Raratonga and Bermuda. The ScS times from these stations all show large delays relative to the average "normal continental" times; the average residual is close to +5 s.

There is some doubt as to whether or not the mantle beneath these oceanic island stations is truly "normal". After all, islands are by definition anomalous, and many may be the sites of convective upwelling in the mantle^{19,20}. A further test for the existence of large oceanic delays is provided by the observation of time differences between the arrivals of ScS and ScSScS, an S phase which is twice reflected off the core and once reflected off the surface. ScSScS–ScS time differences are insensitive to the velocity structures in the vicinity of the source and receiver, as well as to any mislocation errors. By using geometries where the surface bounce of ScSScS occurs beneath a deep ocean basin, vertical travel-time differences beneath continents and normal ocean can be obtained. An analysis of 64 ScSScS–ScS time differences has yielded one-way vertical travel-time differences between oceans and continents which average +3 to +4 s (S. A. Sipkin and J.H.J., unpublished). Although somewhat less than the average delay from the island stations, these oceanic delays are compatible with the times inferred from free oscillations.

When combined with the regional surface-wave dispersion data, the large vertical travel-time differences between continents and oceans imply that continent–ocean heterogeneity extends to depths exceeding 400 km^{13,18}. The velocity

Fig. 2 A histogram plot of ScS residuals for each of Sipkin and Jordan's¹⁸ tectonic classification types. The residuals have been formed by subtracting the average normal continental residual for each earthquake studied, a procedure which eliminates errors due to hypocentral mislocation.



differences in the upper 400 km of the mantle are reasonably well constrained by the surface-wave data, and these differences can account for only a second or two of the travel time difference. To satisfy the observed oceanic delay of +3 to +5 s requires the existence of deeper velocity differences.

This inference is supported by the free oscillation results. The models derived from the free oscillation data are typically characterised by lower shear velocities in the depth range 400–700 km than models derived from continental body wave data¹³.

A sketch of possible shear velocity profiles for shield and oceanic upper mantles is given in Fig. 3. These velocities are tentative; it is unclear exactly how the velocity differences are distributed with depth. For example, it is not known if the depths of the major discontinuities are displaced. Analysis of internally reflected waves shows some promise of answering questions such as these²¹, but the precise delineation of oceanic upper mantle structure must await long-line refraction studies using seismometers on the ocean bottom.

The comparisons made here contrast the "typical" structures of the continents and the oceans. Clearly, important variations in mantle structure exist within the confines of the continental margins and the ocean basins. In North America at least, the seismic data suggest that some or even most of these regional variations are associated with the transition from shield to oceanic structure; that is, much of the continent-ocean transition occurs in regions overlain by continental crust. On a transit south or south-west from the Canadian shield there is a general increase in P and S station anomalies¹ and the appearance of a well developed low velocity zone². Across the Rocky Mountain Front this transition is quite abrupt, and the entire western United States may be underlain by mantle that is essentially oceanic in character. It is important to chart the shield-ocean transition across a quiescent continental margin, such as the eastern coast of North America, again, an exercise requiring seismometers on the ocean bottom. Across such a margin

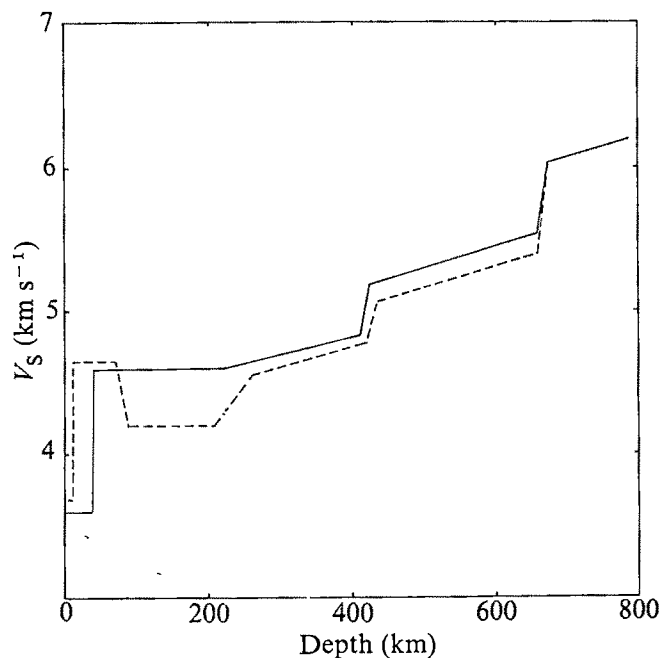


Fig. 3 Possible models of the shear-wave velocity structures beneath continents and oceans. The models are compatible with the surface-wave dispersion data and have a difference in one-way vertical travel time of 3.1 s. —, Shield; ---, ocean.

the lateral velocity gradients may be very large in magnitude.

I have presented elsewhere¹³ a possible model for the upper mantle which can account for the existence of deep-seated continent-ocean velocity contrasts and which is consistent with our knowledge of the upper mantle thermal regime. Briefly stated, this hypothesis postulates that,

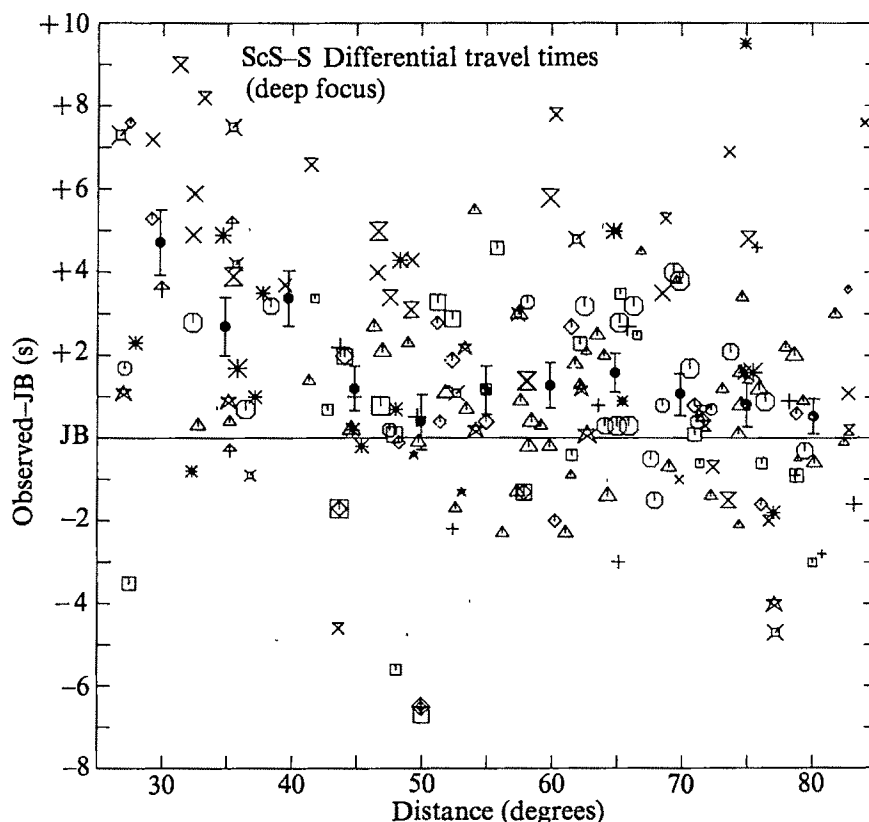


Fig. 4 ScS-S residuals at WWSSN stations from a global distribution of deep-focus events²⁰. Black dots are 5° cell means; error bars represent 1 s.e.m. Each symbol corresponds to a different event, and the size of the symbol is proportional to an assigned subjective quality of the reading.

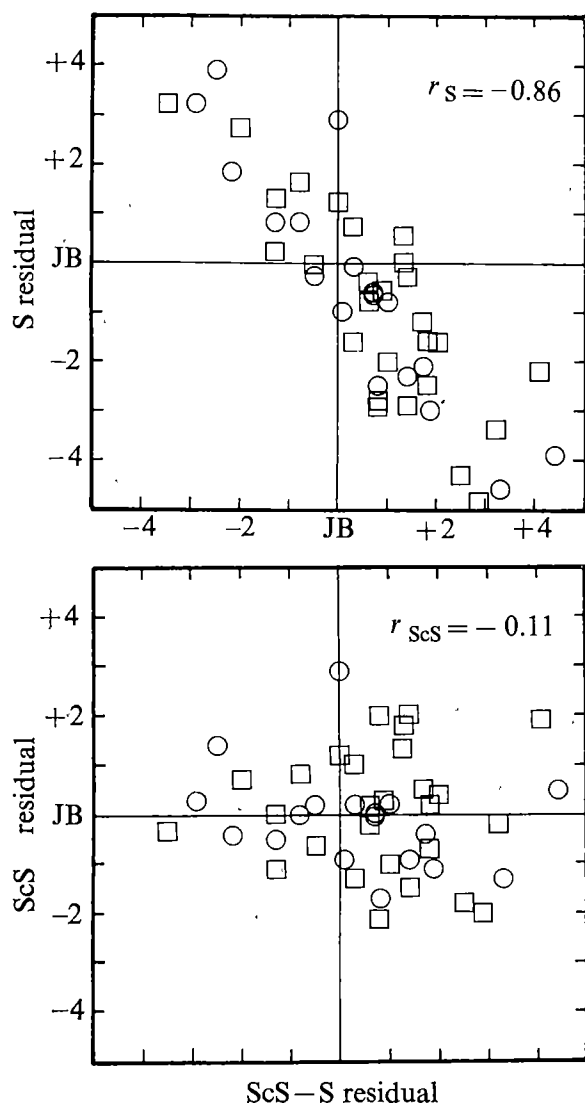


Fig. 5 Correlation plots of S residual versus ScS-S residual (top), and ScS residual versus ScS-S residual (bottom) for times from two Peru-Brazil deep-focus events recorded at United States and Canadian stations²⁸. r_s and r_{scS} are the respective sample correlation coefficients.

beneath the ancient shields, the region which translates coherently in the course of horizontal plate motions (the tectosphere) extends to at least 400 km depth and may occupy the entire upper 700 km of the mantle. Within the continental tectosphere the temperatures are lower, the thermal gradients are super-adiabatic, and the dominant mechanism of heat transport is conduction, not advection. To stabilise the vertical super-adiabatic temperature gradients beneath the shields and the horizontal temperature gradients within the transition from shield to oceanic structure, the model invokes compositional gradients, regions of lower potential temperature consisting of material with intrinsically lower densities.

Admittedly, this model entails a radical departure from the accepted assumption that the thickness of the plates and the lithosphere are identical, it rejects the hypothesis that the continents are decoupled from the mantle at the classical lithosphere-asthenosphere boundary. Furthermore, if the model is correct, continental drift is no longer a simple consequence of plate tectonics. But, in spite of these uncomfortable implications, the model does not seem to be at variance with any significant constraint and thus deserves at least tolerant consideration.

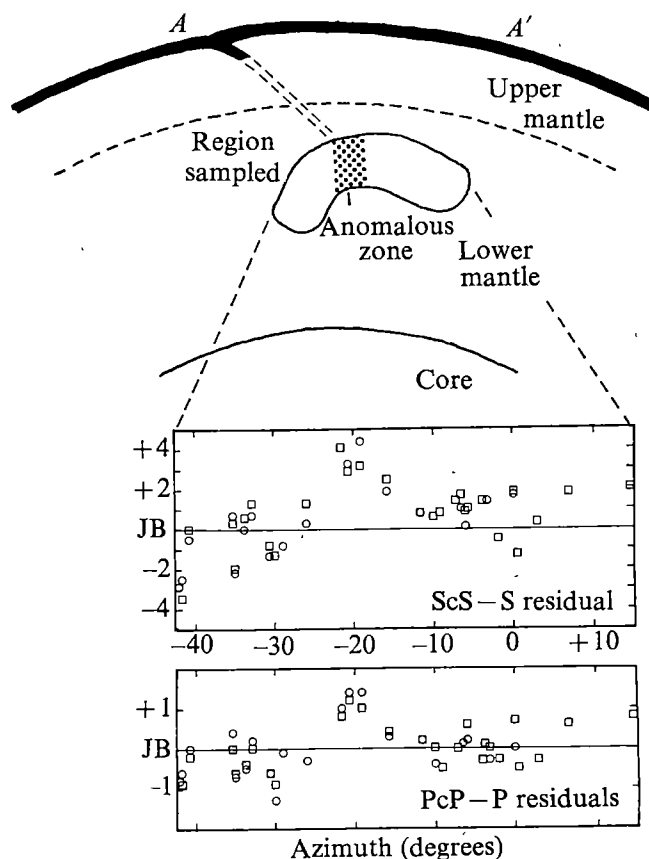
In any case, the seismic data presented here, as well as the old problem of the differences in heat fluxes from the sub-continental and sub-oceanic mantles, require some explanation. The only alternatives I have been able to envisage demand unusual dynamical configurations that seem even more improbable than the tectospheric model.

Lower mantle heterogeneity

Although lateral variability in upper mantle velocities has been known to exist for some time, the existence of lower mantle lateral heterogeneity has been only recently demonstrated. Of course, the difficulty with seeing deep heterogeneity is that it is obscured by geographical variations near the surface, which are generally of larger magnitude in terms of seismic effects. The important work on the subject has been accomplished using a variety of high-resolution techniques, which include body-wave amplitude analysis²², the use of large-aperture seismic arrays²³⁻²⁶, as well as the more conventional measurement of travel times^{27,28}. The most significant result for mantle dynamics to emerge from these studies is the detection of variations in the deep mantle beneath subduction zones.

Data that are particularly suitable for the study of lower mantle structure are the time differences between direct and core reflected phases (PcP-P and ScS-S). At distances greater than 30° or so these differential travel times are not much affected by even gross velocity variations in the crust and uppermost mantle, and they are relatively insensi-

Fig. 6 ScS-S and PcP-P residuals as a function of azimuth from two Peru-Brazil events²⁸. The vertical section through the mantle shows that the large positive residuals in the azimuth window from -15° to -25° define an anomalous zone which lies along the projection of the Benioff zone beneath the Middle America Trench. The section is located on a map view in Fig. 7.



tive to event mislocations as well. Hales and Roberts²⁸ presented 30 observations of ScS-S differential travel times to estimate the radius of the core-mantle boundary. In this study they noticed that the differential travel times showed an unexpectedly large scatter (about 10 s). They speculated that this scatter was due to lateral heterogeneities in the lower mantle, although they did not rule out the possibility of mislocation errors or variations near the source.

I have obtained a considerably larger set of ScS-S times from World-Wide Standard Seismographic Network (WWSSN) recordings of a global distribution of deep-focus events for use in gross Earth modelling^{15,30}. These observations, presented in Fig. 4, show a scatter similar to those obtained by Hales and Roberts. The use of deep-focus events eliminates the near-source traverse through the upper mantle and lends additional support to the hypothesis that the dispersion of times results from velocity heterogeneity in the lower mantle.

A detailed examination of differential travel times, both PcP-P and ScS-S, from two deep focus events in the Peru-Brazil region of South America²⁸ elucidates further the source of the scatter. First, a strong correlation between PcP-P and ScS-S times exists for these events, suggesting the scatter is not simply due to reading errors or some other random noise process. Second, the ScS-S differential times show a strong negative correlation with absolute S times and do not correlate with ScS times (Fig. 5). This implies that the scatter arises from variations along the path of the direct phase, not the core reflection. (This was part of the rationale for using ScS to probe upper mantle heterogeneity, discussed above.) If this interpretation is generally correct for ScS-S, the magnitude of the scatter in these times can be used to estimate the degree of lower mantle heterogeneity. The times in Fig. 4 show a total variation of about 8 s. Supposing the typical scale length for lower mantle heterogeneity to be 1,000 km (an arbitrary number), we obtain velocity contrasts along the path of the S wave of about 5%, a surprisingly high value.

A third result found in the study of the Peru-Brazil data was that the residuals of both PcP-P and ScS-S show a coherent azimuthal dependence (Fig. 6). Of particular note is the grouping of large residuals in the azimuth window -15° to -25° . The rays corresponding to these anomalous times traverse the lower mantle through a region which lies along the extension of the Benioff zone beneath the Middle America Trench, as illustrated in Fig. 6. The existence of an anomaly at approximately this location is supported by two other observations. Davies and Capon³¹ reported anomalous body-wave multipathing at the Large-Aperture Seismic Array (LASA) in Montana for a northern Columbian event, which they suggested might be due to lower mantle heterogeneity. The great-circle ray for this earthquake passes through the anomalous zone defined by the Peru-Brazil data (Fig. 7). In a separate study, Sheppard³² presented an array diagram for the Norwegian Seismic Array (NORSAR), and he noted the existence of a probably source-related anomaly for earthquakes in central America. Events in the Oaxaca district of Mexico separated by as little as 75 km in actual location show array mislocations of as much as 700 km. The magnitude of this splitting must mean that the velocity heterogeneity responsible extends into the lower mantle. Again, the ray paths lie near the anomalous zone delineated by the differential travel-time data (Fig. 7).

Not only do the differential travel times define the location of the anomalous zone, but the positive residuals indicate that the anomaly is characterised by unusually high velocities for both P and S waves. To explain this feature as a thermal anomaly, we found that temperature contrasts of at least 200°C were required, the anomaly being cooler than the surrounding mantle. Thus, the interpretation suggested by these data, and adopted by Jordan

and Lynn is that this high velocity anomaly beneath the Caribbean marks the site of cold material descending in a convecting mantle. The implication of this statement is that the lithospheric slab, or some portion of the slab and its adjacent mantle, penetrates to a depth which exceeds 800 km and which may be greater than 1,400 km.

Evidence is accumulating for deep lateral structure beneath other subduction zones, although the results are less decisive than those for the study described above. Weichert²³ has attributed certain $dT/d\Delta$ and azimuth anomalies for P waves observed at the Yellowknife array to lateral velocity gradients near 800 km depth beneath the Aleutian Arc. Davies and Sheppard²⁴ in their analysis of the LASA array diagram noted that significant changes in the mislocation vectors for some western Pacific regions correlated with transitions from one arc to another, suggesting the anomalies were due to a structure near the source structure. Powell (unpublished), using LASA and two other large arrays in the western United States, has found that this correlation also exists for other regions, notably the Tonga-Fiji Arc. She has used ray-tracing

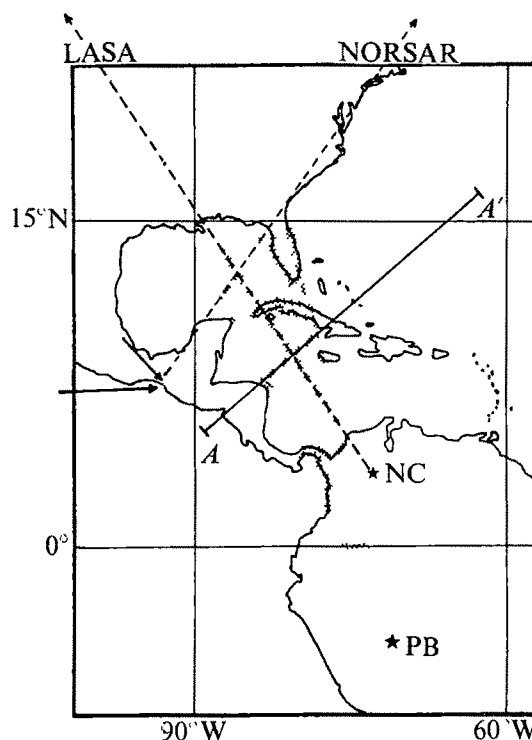


Fig. 7 Map of the Caribbean area. Shaded region is the surface projection of the mantle below 600 km traversed by rays with large positive residuals from two Peru-Brazil (PB) events²⁷. Also shown are the great-circle path between a northern Columbian (NC) event and LASA, for which Davies and Capon³¹ have observed body-wave multipathing, and the great-circle path between two Mexican events and NORSAR, which show large differences in array mislocation. The arrows are the NORSAR mislocation vectors for these earthquakes³².

techniques to investigate the sources of the anomalies and concludes that lateral velocity variations are present at depths exceeding 800 km. Engdahl³³ has observed travel-time differences at Alaskan stations from deep-focus events in the Tonga-Fiji region which are anomalously large and which require that the plate effects extend deeper than 650 km.

The conclusion that descending lithospheric slabs penetrate deep into the mantle cannot be disputed on

theoretical grounds. A numerical study by Schubert, Yuen and Turcotte³⁴ has demonstrated that temperature contrasts of 700 °C can exist between the slab and the adjacent mantle at 800 km depth, which is consistent with the constraints established in the Caribbean study.

Lateral structure in the mid-mantle beneath subduction zones is perhaps sufficient to explain the scatter in ScS-S times shown in Fig. 4. This hypothesis cannot, however, account for all of the heterogeneity in the lower mantle. Julian and Sengupta²⁷ have found a significant increase in P-wave travel-time residuals at epicentral distances beyond 70° which seems to be associated with lateral structure at depths greater than 2,600 km. These variations show no obvious correlation with surface tectonics.

Actually, the lowermost mantle, which includes Bullen's "region D", has been known to be laterally variable for some time. Phinney and Alexander²² reported geographical variations in the amplitude decay of core-diffracted P waves, a measurement quite sensitive to the velocity structure near the core-mantle boundary.

Array studies³⁴ also indicate the presence of heterogeneity in this region, but the problems with eliminating near-source effects are severe. For example, Kanasewich and others³⁵ saw $dT/d\Delta$ and azimuth anomalies at Canadian arrays and at LASA which they ascribed to a feature near the core-mantle interface beneath Hawaii. (These anomalies were also noted by Davies and Sheppard, ref. 24.) The observation provoked much excitement, since it seemed to confirm a deep mantle source for the Hawaiian hotspot, first suggested by Wilson and Morgan¹⁹. But a recent study by Okal and Kuster³⁶, who looked at the deep mantle beneath Hawaii using the French Polynesian array, failed to support this hypothesis, and Powell (unpublished) has shown that the observations reported by Kanasewich and his colleagues probably result from near-source structure of the sort described above.

Nevertheless, the mantle directly above the core-mantle boundary seems to be quite inhomogeneous, probably on many scales. Julian and Sengupta found lateral correlations in residuals with scales on the order of 1,000 km. Much smaller scale heterogeneity in this region (scale lengths on the order of tens of kilometres) has been postulated by Haddon *et al.*³⁷ to account for the unusual characteristics of certain arrivals precursory to the phase PKIKP.

The relationship of this heterogeneity near the core-mantle interface to mantle dynamics remains obscure.

Implications of lateral heterogeneity

Although the formal study of the Earth's lateral heterogeneity is still in its infancy, two conclusions with profound implications for mantle dynamics seem to be required by the available data. (1) Contrasts in the seismic velocity profiles beneath the stable continental shields and the ocean basins persist to depths exceeding 400 km and may extend as deep as 700 km. (2) Strong lateral velocity gradients exist at depths greater than 800 km beneath many subduction zones. Beneath at least one of these, the Middle American Trench, is a zone of anomalously high seismic velocity which extends to perhaps 1,400 km depth.

I interpret these results in terms of the following hypotheses. (1) Beneath the ancient shields the mass which translates coherently in the course of horizontal plate motions (the tectosphere) is at least 400 km thick. Within this tectosphere, the temperatures are lower, the thermal gradients are super-adiabatic, and the dominant mechanism for heat transport is conduction. Anomalous temperature gradients are dynamically stabilised by compositional gradients¹². (2) The lithospheric slab descending beneath subduction zones (and probably some of its adjacent mantle)

penetrates well into the lower mantle before being thermal equilibrated. These, of course, are only hypotheses formulated from limited data, and they must be vigorously tested before being taken too seriously.

Should either prove to be correct, however, one implication may be drawn which constrains the nature of mantle dynamics: more than just the Earth's crust and upper mantle participates in whatever form of convection is responsible for plate motions. Some sort of deep-mantle convection would be necessary to accommodate the mass flow beneath the continents required by the current geometry of seafloor spreading and destruction, given that the tectosphere is very thick beneath continents. If slabs penetrate the lower mantle, then the implication that deep circulation exists is immediate: we have observed it directly.

Deep-mantle convection is not a radical concept on geophysical grounds, but it does pose a geochemical problem. It has been recognised that the oxidation state (as measured by the ferric-ferrous ratio) and nickel content of rocks seen at the Earth's surface are much larger than would be expected if upper mantle material had even been in equilibrium with the metallic core³⁸⁻⁴⁰. If convective cycling involved material transport at the base of the mantle, in the present epoch or those past, then these observations would be very difficult to explain. On these grounds alone, it may be necessary to postulate the existence of a barrier which prevents the exchange of iron and nickel between the upper mantle and the core. On the basis of the seismic data, however, it is unlikely that this barrier is the entire lower mantle.

As indicated in this report, progress in delineating the Earth's lateral heterogeneities is proceeding rapidly. Much work remains to be done, but it is hoped that the study of lateral variations will provide the keys for unlocking the secrets of mantle dynamics.

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articles

X-ray analysis of glucagon and its relationship to receptor binding

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X-ray analysis of the pancreatic hormone glucagon shows that in crystals the polypeptide adopts a mainly helical conformation, which is stabilised by hydrophobic interactions between molecules related by threefold symmetry. A model is presented in which the glucagon molecule exists in dilute solutions as an equilibrium population of conformers with little retention of structure, and in which the helical conformation is stabilised by hydrophobic interactions either as an oligomer or as a complex with the receptor.

GLUCAGON is a polypeptide hormone of 29 amino acids (ref. 1 and Fig. 1) which is synthesised and stored in the α cells of the islets of Langerhans of the pancreas. The hormone activates glycogenolysis and gluconeogenic pathways resulting in raised blood glucose levels. Rodbell *et al.*² showed that this is mediated by specific binding to a plasma membrane receptor site on the regulatory component of adenylate cyclase of liver and other cells, which gives rise to an increase of intracellular levels of the second messenger cyclic AMP.

An understanding of the nature of the glucagon-receptor interactions depends critically on a proper description of the conformation of the hormone when bound to the receptor. Solution studies show that glucagon exists as an equilibrium population of conformers in dilute aqueous solution^{3,4} but the structure becomes more ordered on self association^{5,6} and in the presence of detergents⁷ or lipid micelles⁸. Preliminary X-ray studies^{9,10} indicated that the crystal structure is mainly helical. We have now carried out a full X-ray analysis at ~ 3.0 Å resolution using the method of isomorphous replacement and anomalous scattering. We describe here our findings on the crystal structure of glucagon; we discuss the relevance of this structure to solution studies, to storage in α cells and to interactions with the receptor; and we compare the structure of this hormone with that of insulin, the only other polypeptide hormone whose structure has been determined by X-ray analysis¹¹.

Glucagon crystals

Glucagon was first crystallised by Staub *et al.*¹² and the space-group ($P2_13$) and unit cell parameters ($a = 47.91$ Å) of the cubic crystals were reported by King⁹. We found that large crystals (> 1 mm diameter) could be grown at pH 9.2 in phosphate buffer by warming a 0.3% solution of glucagon to

50 °C and cooling slowly in a Dewar packed in a hot-box. As pH 9.2 is higher than physiological pH (~ 7.4), we studied the stability of the crystals as a function of pH. Transfer of the crystals to a buffer (acetate or phosphate) in the pH range 5.8–7.5 leads to the cracking of the crystals, but not to their complete disintegration. The crystals still diffract X rays; the maximum resolution (~ 3 Å) of the data is not affected but the mosaic spread is increased. The unit cell dimensions are decreased ($a = 47.1$ Å) and the diffraction pattern shows large intensity changes. Crystals identical to these, but uncracked, can be grown from dilute solutions of glucagon at pH 6.0, but the decreased solubility of glucagon at this pH makes it difficult to grow large crystals. These experiments suggest that the molecular structure is flexible in the crystals as well as in solution.

This made us cautious in our attempts to prepare heavy atom derivatives. W. N. Lipscomb (unpublished) noticed changes in intensities which indicated changes in structure at high pH when heavy atom salts were added. We have shown that metal cations such as Ag^+ and Hg^{2+} and complexes such as PtCl_4^{2-} change the structure at pH 9.2 towards the lower pH crystal form; but many other anionic complexes such as $\text{Au}(\text{CN})_2^-$ and $\text{UO}_2\text{F}_6^{3-}$ cause a change in the reverse direction. Such changes undoubtedly account for the problems encountered by previous workers using the method of isomorphous replacement.

Determination of the structure

We decided to attempt the X-ray analysis of crystals soaked in acetate buffer in the pH range 5.6–7.5. This was for three reasons. First, the pH is closer to physiological pH than at pH 9.2. Second, we found the structure less susceptible to small changes of pH than at pH 9.2. Finally, acetate anions do not form insoluble complexes with metal cations such as Ag^+ , UO_2^{2+} and Sm^{3+} ; on the other hand, these cations have insoluble phosphates and form insoluble hydroxides at pH 9.2.

Soon after our preliminary studies on heavy atom soaking and pH dependence of the crystal structure, W. N. Lipscomb of Harvard University sent us a summary of his X-ray studies in the pH range 9.5–8.5 which we found very helpful. Three heavy atom derivatives using Ag^+ , PtCl_4^{2-} and $\text{Pt}(\text{NO}_2)_4^{2-}$, respectively, were prepared using the conditions shown in Table 1. The three derivatives gave similar but not identical intensity changes in the diffraction patterns. Ag^+ gave a highly isomorphous derivative but the platinum derivatives gave rise to small changes of cell dimensions and the data were attenuated at interplanar spacings of $d < 3.5$ Å.

Three dimensional X-ray data were collected on a Hilger and Watts four-circle diffractometer using a new method for calculation of integrated peak intensities of reflections¹³. For the native and PtCl_4^{2-} glucagon crystals three equivalents

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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
His	Ser	Gln	Gly	Thr	Phe	Thr	Ser	Asp	Tyr	Ser	Lys	Tyr	Leu	Asp	Ser	Arg	Arg	Ala	Gln	Asp	Phe	Val	Gln	Trp	Leu	Met	Asn	Thr
									Trp	Gln	Val	Phe	Asp	Gln	Ala	Arg	Arg	Ser	Asp	Leu	Tyr	Lys	Ser	Tyr				
									25	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10				

Fig. 1 Sequence of porcine glucagon after Bromer *et al.*¹. Residues 10–25 are written in reverse to show the approximate twofold symmetry of this part of the sequence.

(*hkl*, *lhk* and *klh*) were measured, but for the Ag^+ and $\text{Pt}(\text{NO}_2)_4^{2-}$ glucagon crystals three Friedel equivalents *hkl*, *lhk* and *klh* were also measured to estimate anomalous differences (ΔI). The data were corrected for Lorentz and polarisation effects and absorption corrections were made by the empirical method of North *et al.*¹⁴. Isomorphous difference, anomalous difference and F_{HLE}^2 Patterson functions were computed. F_{HLE}^2 values^{15,16} were given by

$$F_{\text{HLE}}^2 = F_P^2 + F_{\text{PH}}^2 - 2 \left[F_{\text{PH}}^2 F_P^2 - \frac{k^2}{4} (\Delta I)^2 \right]^{\frac{1}{2}}$$

where F_P and F_{PH} are the structure factor amplitudes for the native and derivative crystals and k is the ratio of the real and imaginary parts of the heavy atom scattering factor, and was determined empirically¹⁷. The Harker peaks corresponding to each heavy atom site (Table 1) were identified and the atomic positions were refined using conventional least squares refinement with coefficients $|F_{\text{PH}} - F_P|$ for centric zones, and F_{HLE} for the acentric zones of the Ag^+ and $\text{Pt}(\text{NO}_2)_4^{2-}$ glucagon derivatives. Standard deviations (σ) were estimated for the coefficients and only data greater than 1σ were used. The final residuals are given in Table 1. Difference maps with coefficients $(F_{\text{HLE}} - F_{\text{H}}(\text{calc})) / \alpha_{\text{H}}(\text{calc})$ were used to check for minor heavy atom sites. Refinement using lack of closure procedures was not attempted as the positions of the heavy atoms in the derivatives were strongly correlated.

Phases were first calculated using single isomorphous replacement and anomalous scattering for each of the Ag^+ and $\text{Pt}(\text{NO}_2)_4^{2-}$ derivatives. The correct hand of the heavy atom arrangements was established by calculating difference Fourier for a second derivative using phases calculated for each of the possible enantiomorphic heavy atom arrangements of the first and observing the peak heights at the heavy atom positions of the second. Difference Fourier techniques were also used to relate different heavy atom sets to the same origin.

Phases were calculated using the method of isomorphous replacement incorporating anomalous scattering¹⁸. The figure of merit was 0.84 for the data within the 4.5 Å sphere but decreased steeply especially in the range $3.5 > d > 3.0$ Å where the data were weak and only two derivatives were included. The mean figure of merit was 0.70. The best phases¹⁹ were used to calculate an electron density map, part of which is shown in Fig. 2.

Crystal structure

The electron density map shows well defined helical regions and large volumes of low density which correspond to regions of solvent. Comparison of the electron density with a molecular model using an optical device²⁰ shows that the helical regions correspond to about sixteen residues of α helix, extended at either end by four residues of less regular, right-handed helix. The density of the α -helical region shown in Fig. 3 corresponds well with residues 10–25 of the glucagon molecule. The aromatic sidechains of tyrosine 10, tyrosine 13, phenylalanine 22 and tryptophan 25 are clearly defined although the polar sidechains

Table 1 Data for refinement of the positions of heavy atoms in the derivatives of glucagon in different resolution ranges

		No. of Sites	Z	x	y	z	∞ –4.5 Å				4.5–3.5 Å				3.5–3 Å					
							$\overline{F_{\text{P H}}}$	$\overline{F_{\text{H}}}$	E_{iso}	R_{C}	$R_{\text{F}_{\text{HLE}}}$	$\overline{F_{\text{P H}}}$	$\overline{F_{\text{H}}}$	E_{iso}	R_{C}	$R_{\text{F}_{\text{HLE}}}$	$\overline{F_{\text{P H}}}$	$\overline{F_{\text{H}}}$	E_{iso}	R_{C}
10 mM AgNO ₃ Acetate buffer 2 d	1	33	−0.165	−0.067	0.186	175	57	28	0.38	0.37	122	33	22	0.58	0.50	72	22	14	0.60	0.47
10 mM K ₂ PtCl ₄ Acetate buffer 18 d	2	8	−0.122	−0.069	0.198															
		8	−0.075	−0.047	0.182	172	35	19	0.44		110	34	21	0.51		89	35	25	0.59	
10 mM K ₂ Pt(NO ₂) ₄ Acetate buffer 21 d	1	78	−0.129	−0.079	0.178	216	133	60	0.37	0.33	141	111	54	0.54	0.43					

$\overline{F_{\text{PH}}}$ and $\overline{F_{\text{H}}}$ are the mean values of the structure factor amplitudes for the derivative structure and the heavy atom constellation, and E_{iso} is the root mean square lack of closure for isomorphous differences for the best phase angle. Z, the occupancy in electrons, and x, y and z the fractional coordinates for each heavy atom position were obtained by least squares F_{HLE} refinement for the Ag^+ and $\text{Pt}(\text{NO}_2)_4^{2-}$ glucagon derivatives, and by refinement against isomorphous differences on the centric zones for the PtCl_4^{2-} derivative. The residuals are defined as

$$R_{\text{C}} = \frac{\sum |F_{\text{PH}} - F_{\text{H}}| - F_{\text{H}}(\text{calc})|}{\sum |F_{\text{PH}} - F_{\text{H}}|}$$

for centric zones and

$$R_{F_{\text{HLE}}} = \frac{\sum |F_{\text{HLE}} - F_{\text{H}}(\text{calc})|}{\sum F_{\text{HLE}}}$$

for all reflections. F_{HLE} is defined in the text.

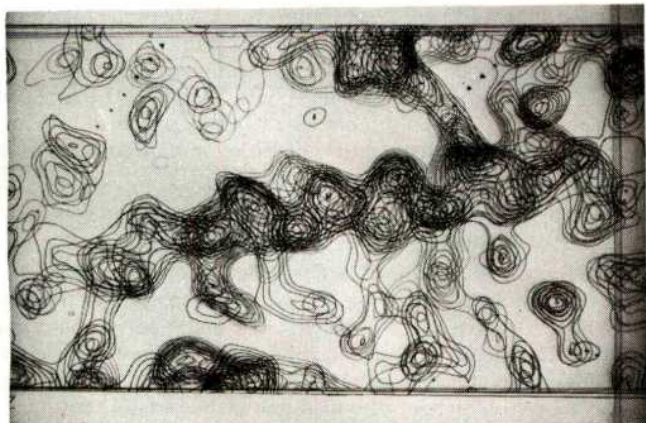
of arginine and lysine have weaker density. In an α -helical conformation, residues 10–25 have a very approximate twofold symmetry as indicated in Fig. 1 and so the helix fits tolerably, but less well if the direction of the polypeptide is reversed. The correct chain direction was confirmed by the better fit of tryptophan 25, by the well defined electron density of phenylalanine 6 and the continuous density corresponding to residues 1–5 in a non-helical conformation.

Two observations suggest that the polypeptide chain of residues 1–5 is flexible: the density of residues 1–5 is weaker than that of the helix and preliminary studies on the pH 9.2 crystal form indicate changes in the conformation of these residues. Change of pH from 9.2 to around 6 will almost certainly lead to protonation of the amino terminus, and probably formation of an ion pair with the carboxy terminus of the glucagon molecule in the next unit cell. This may be the explanation for the rearrangement indicated in the crystals at pH 9.2. The metal ions Ag^+ , PtCl_4^{2-} and $\text{Pt}(\text{NO}_2)_4^{2-}$ bind between methionine 27 and the N terminus, and their substitution at this position probably induces changes in conformation and crystal packing, similar to those induced by lowering the pH.

The conformations of the non-polar residues in the regions 6–14 and 22–27 are shown in Fig. 3. In the crystal structure they are packed against equivalent residues of other molecules to give hydrophobic regions. The threefold axes of the cubic cell pass close to the glucagon molecule (Fig. 2), and contacts arise between molecules related by these symmetry operations. Figures 4 and 5 show trimeric arrangements of glucagon viewed down the two threefold axes which give rise to extensive intermolecular contacts.

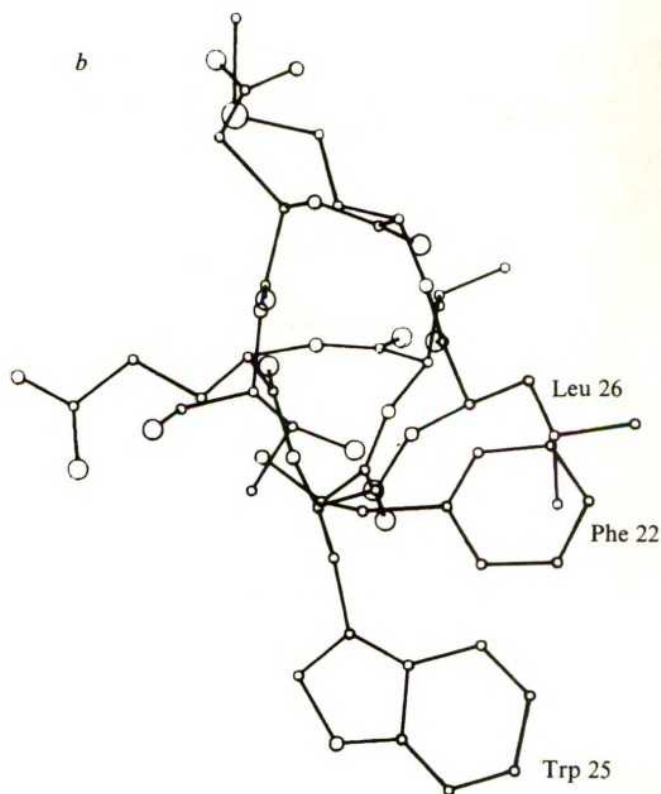
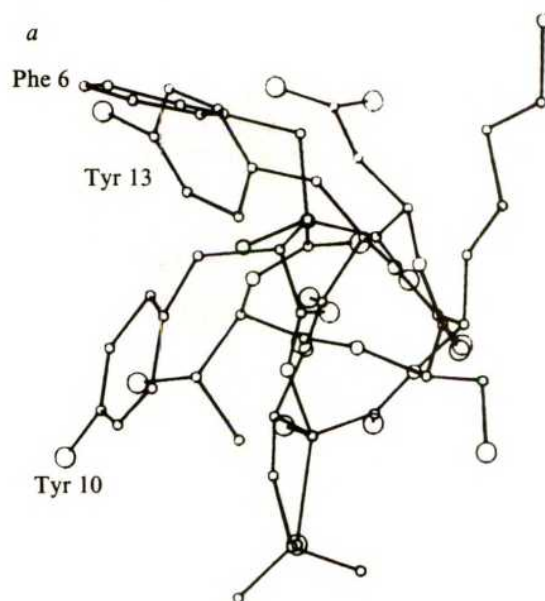
Figure 4 shows the association of the glucagon molecules through contacts between the sidechains of tryptophan 25, leucine 26 and phenylalanine 22 of one molecule and tyrosine 10, tyrosine 13 and phenylalanine 6 of the second. This leads to a triangular structure with the central region around the threefold axis containing solvent and the sidechains of arginines and aspartic acids. In Fig. 5a the association around a second threefold axis leads to contacts between equivalent amino acids at the carboxy terminus of the molecule including phenylalanine 22, valine 23 and leucine 26, which are shown enlarged in Fig. 5b. This hydrophobic region is extended by close packing of sidechains of phenylalanine 6 and tyrosine 10 of other molecules so that the region of non-polar contacts described first is continuous with the second. The result of this complicated cubic close packing is to bury the hydrophobic regions shown in Fig. 3, which are formed when the molecule adopts a helical conformation.

Fig. 2 Part of the $\sim 3 \text{ \AA}$ electron density map viewed along a cell axis. Triangles indicate the positions of the threefold axes in the unit cell.



As King⁹ suggested, the crystal structure approximates to a cubic packing of cylinders; the individual protomers do not have a globular structure which characterises proteins studied by X-ray analysis.

Fig. 3 The conformation of residues (a) 6–14 and (b) 22–29 viewed approximately along the helix axis showing the clustering together of hydrophobic residues.



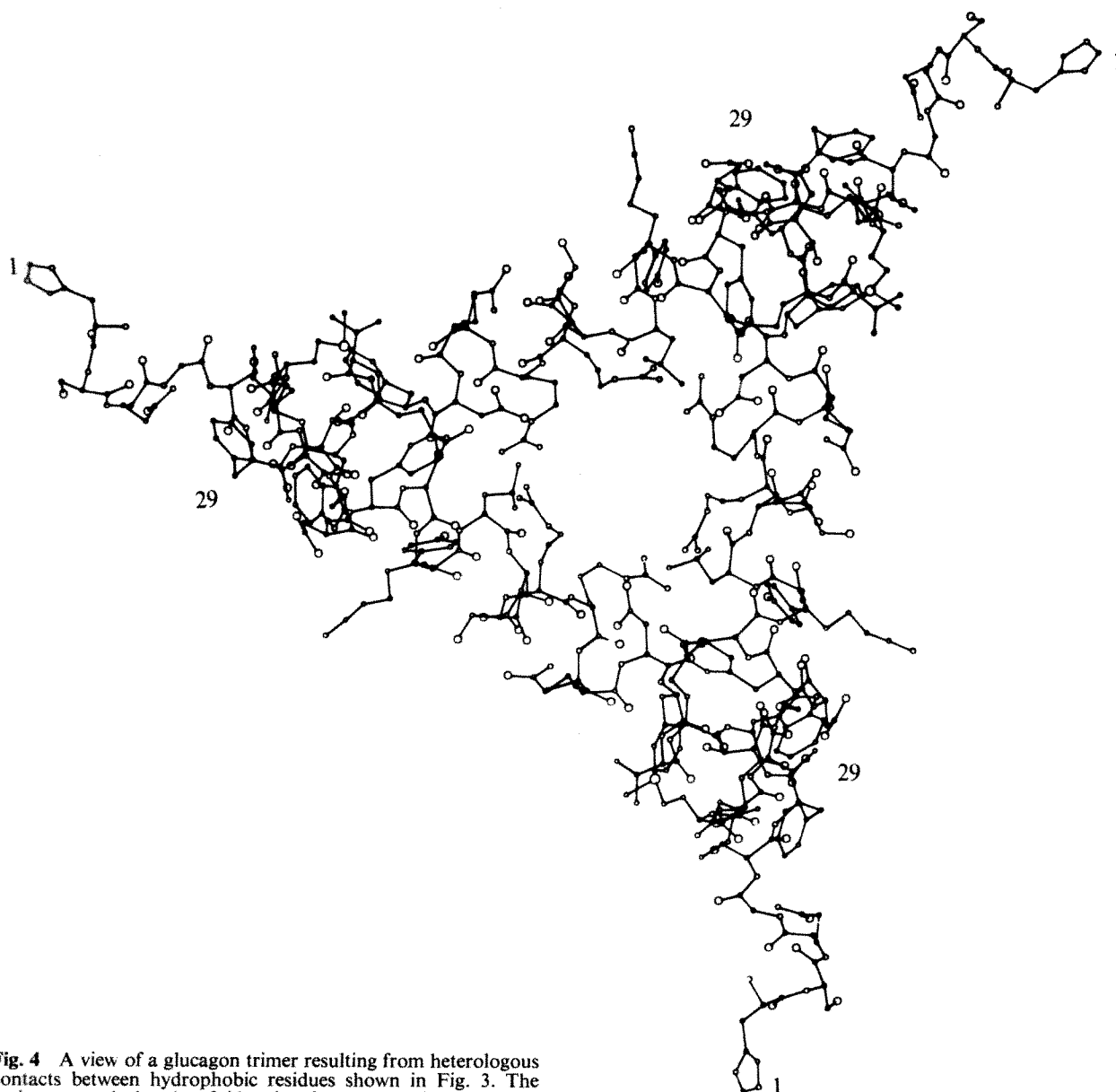


Fig. 4 A view of a glucagon trimer resulting from heterologous contacts between hydrophobic residues shown in Fig. 3. The region around the threefold axis of symmetry in the centre comprises hydrophilic residues including arginines and aspartates.

Solution structure

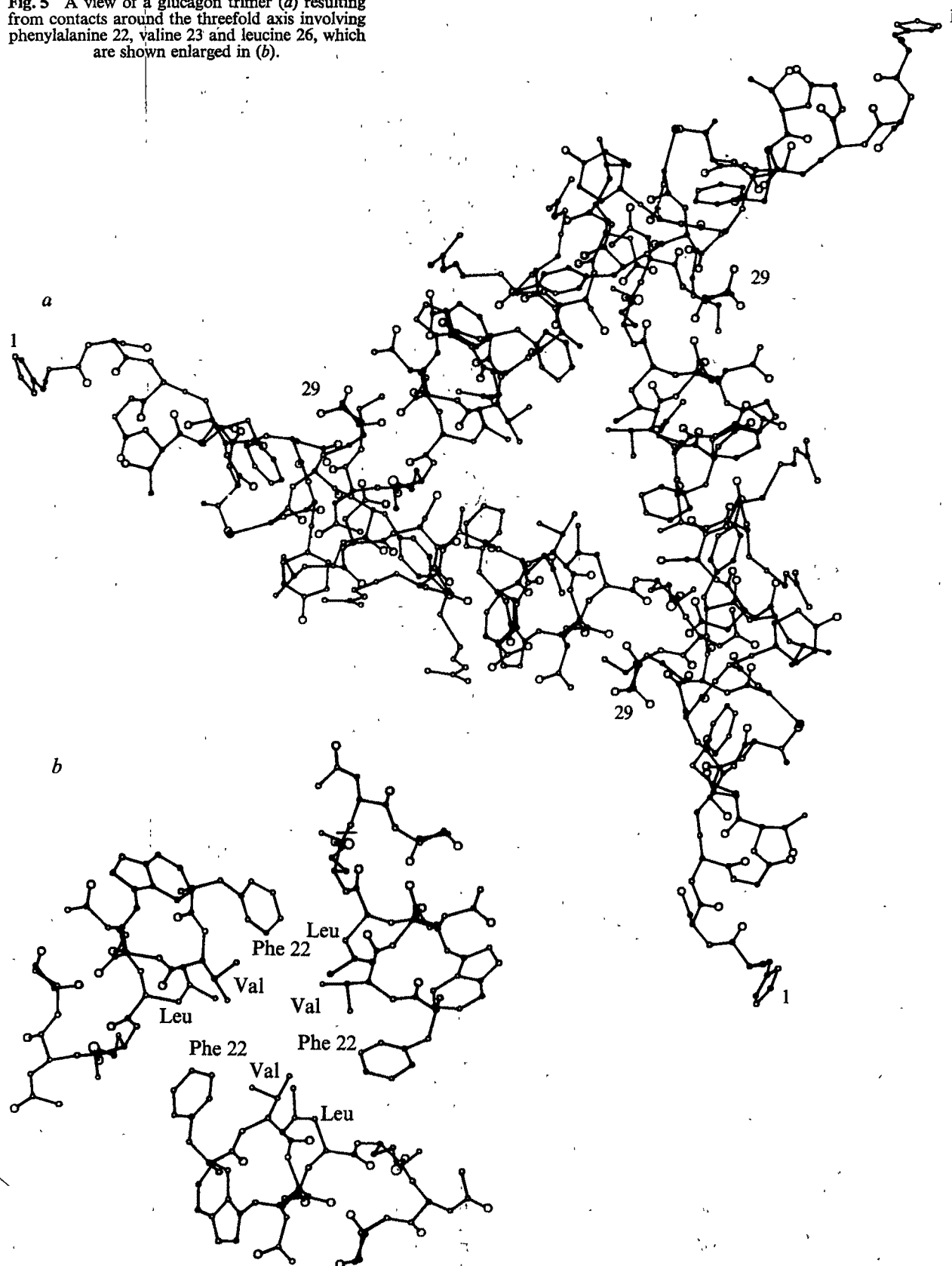
In solution at high dilutions glucagon does not have a highly ordered conformation^{2,3}; it is probably flexible, existing as an equilibrium population of conformers. As a result of the low solubility at neutral pH, most solution studies have been carried out at pH 10. At this pH, optical rotatory dispersion⁴ and circular dichroism⁵ indicate that a largely α -helical conformation is induced by concentrating to 3 mg ml⁻¹, and that the conformational change accompanies an association of molecules probably to trimers^{4,5} and possibly to higher oligomers²¹. The association is accompanied by changes in ellipticity and wavelength in the circular dichroism arising from the tyrosine and tryptophan chromophores⁵. These observations can be explained reasonably well in terms of the crystal structure. The association of monomers to trimers resulting in an α -helical conformation probably corresponds to the formation of the trimer shown in Fig. 4, as this arrangement requires a helical conformation, and unlike the trimer shown in Fig. 5, it would give rise to changes in the near ultraviolet circular dichroism due to both tryptophan and tyrosine. It is also consistent with the observation²² that association affects the *pK* of the phenolic hydroxyl of tyrosine 10, which in the crystal trimer appears to be more buried than tyrosine 13.

The far ultraviolet circular dichroism of the glucagon molecules at pH 10 and 3 mg ml⁻¹ is very similar to the spectrum of zinc insulin hexamers which has about 50% of its residues in right-handed helical conformations¹¹. This percentage is less than that observed in the crystal structure of glucagon. The difference, however, may not be real; it may be a consequence of the rather irregular helix in glucagon. Alternatively it may arise partly from differences in conformation between pH 10 and pH 5.8–7.5 where the crystal structure is stable, and partly from a tightening up of the helix as the trimers are associated to higher oligomers as seen in the crystals. Whatever the explanation, the crystal structure demonstrates why a helical arrangement is favoured by association of glucagon molecules; it leads to burying of hydrophobic residues.

Receptor binding

Rodbell *et al.*²³ have found evidence that discrete regions of the glucagon molecule are important to receptor binding and activity. On the basis of studies with des-histidine-glucagon and smaller fragments of the sequence, they suggest that while essentially the entire molecule of glucagon must be considered to be the active species, the histidine at the amino terminus is relatively unimportant to receptor binding.

Fig. 5 A view of a glucagon trimer (*a*) resulting from contacts around the threefold axis involving phenylalanine 22, valine 23 and leucine 26, which are shown enlarged in (*b*).



At physiological concentrations ($\sim 10^{-11}$ M) glucagon must exist as a monomer and so it is unlikely that glucagon trimers or higher oligomers have any role in receptor binding. But the decreased stability with reduced temperature or with the addition of urea indicates that the glucagon receptor complex is stabilised by hydrophobic interactions²⁴. We are

therefore not concerned with conformation of the monomer in aqueous solution, but rather with the structure stabilised by interaction with a hydrophobic surface. A helical structure is stabilised by lysolécithin⁸, cetyltrimethyl ammonium bromide⁷ and glycols²⁷ which increase the hydrophobicity of the environment of the glucagon molecule. The crystal structure shows that

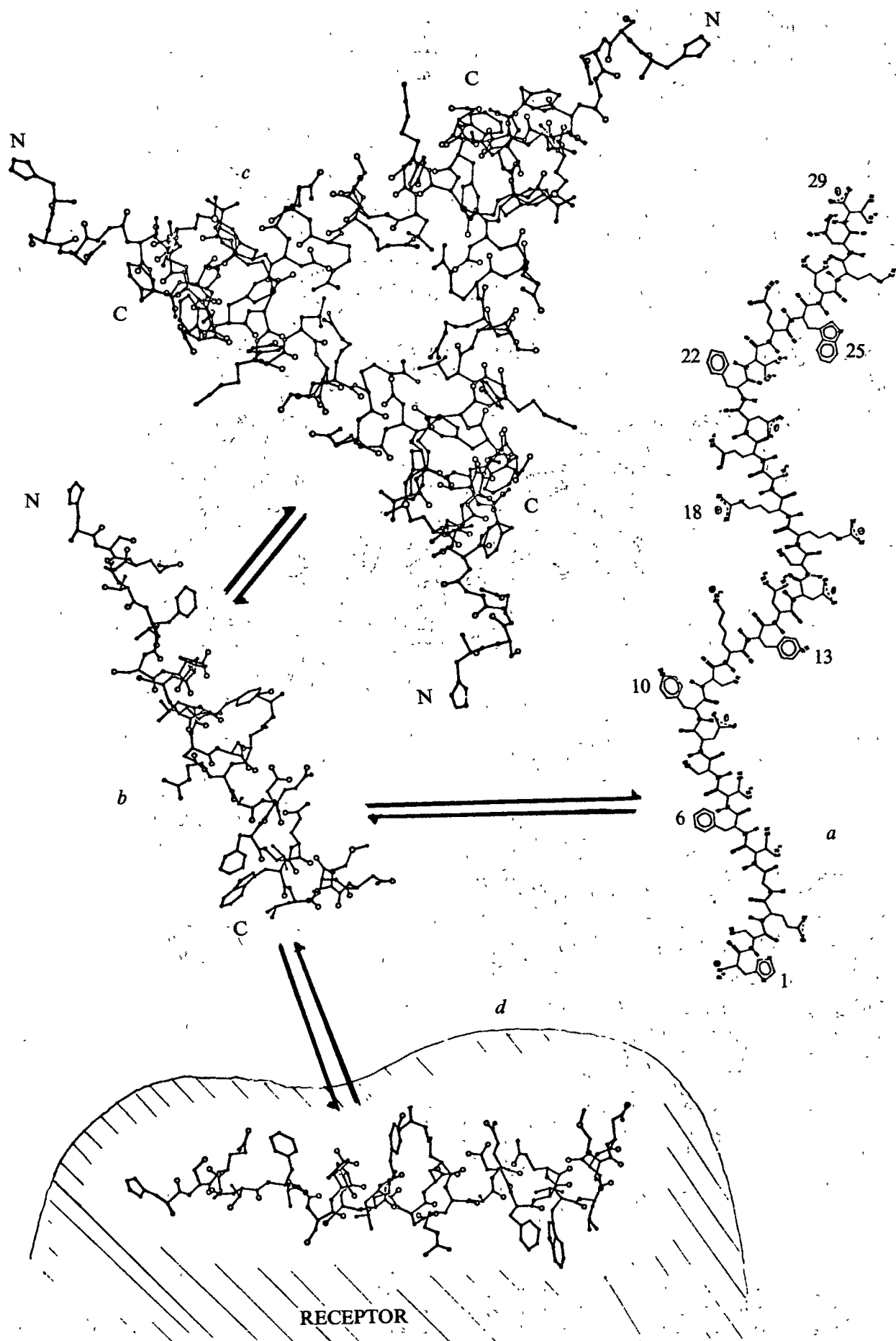


Fig. 6 A schematic representation of the equilibrium between conformers of glucagon. (a) Hypothetical random coil structure in equilibrium with a helical form (b). The helical form is stabilised either as trimers (c) or by association with a receptor (d) by hydrophobic interactions.

a helical conformation is stabilised by association of glucagon molecules to form trimers and higher oligomers. This structure is favoured by hydrophobic contacts because in a helical arrangement non-polar residues are clustered together in two surface regions (Fig. 3). The receptor-bound glucagon may have a similar conformation stabilised by interaction of the glucagon with two hydrophobic regions on the receptor. The importance of both these regions for binding is consistent with the retention of these hydrophobic residues in all active glucagons, the strong receptor binding of des-histidine glucagon, and the inability of either amino-terminal fragments (glucagon 1-21, glucagon 1-23) or carboxy-terminal fragments (glucagon 20-29, glucagon 22-29) to compete with labelled glucagon at its receptor²⁴. The hydrophilic residues adjacent to the two hydrophobic regions may give rise to further stabilising interactions in the glucagon-receptor complex and this may explain the conservation of most of these residues in glucagons from different species. Carbamylation of lysine 12 has little effect on biological activity *in vitro*²⁵; however, modification with a larger group such as trinitrobenzyl²⁶ decreases the biological activity. It also increases the helical content; it probably interacts with the adjacent tyrosine 10 and tyrosine 13, so stabilising the helix but interfering with receptor binding.

This model implies that the glucagon molecule exists in solution as an equilibrium population of conformers with little retention of structure, and that the helical conformation is stabilised by hydrophobic interactions either as an oligomer or as a complex with the receptor, as shown in Fig. 6. This scheme is reminiscent of that of Schwyzer for flexible hormones²⁸. An alternative model would involve an induced fit for the interaction of the glucagon molecule with the receptor²⁹. For instance the C-terminal residues 22-27 containing one hydrophobic area may bind initially in a helical arrangement and induce a helix in the residues 6-21 in a stepwise fashion so that the second hydrophobic area can also bind.

The length of the largely helical glucagon molecule is over 40 Å and the centres of the two hydrophobic regions are about 20 Å apart. This is a very long structure to be bound to one regulatory unit of adenylate cyclase, which may have a molecular weight of about 26,000 (ref. 30) and it is conceivable that glucagon binds two regulatory subunits, each through one hydrophobic region. If these two units are closely homologous and arranged as a dimer with a twofold axis, this would explain the approximate symmetry of the hydrophobic patches around the centre of the helical region shown in Fig. 1. The hydrophobic groups in the centre may form further stabilising interactions with the receptor in the region between the regulatory subunits. A similar twofold symmetry has been proposed for the active conformation of luteinising hormone releasing hormone³⁰, and it has been suggested that this hormone binds two symmetry related subunits in a similar way to the binding of the allosteric effector 2,3-diphosphoglycerate to haemoglobin.

In the crystal structure the region 1-4 does not form a well defined helical arrangement, but changes its conformations easily. This may be facilitated by the presence of glycine 4; glycine can easily attain conformations which are energetically unfavourable to amino acids with sidechains. It is possible that the flexibility also has a role in biological activity. Once the helical region is bound to the receptor the residues 1-4 may change in conformation to interact with the receptor in a way which gives little further thermodynamic stability to the glucagon-receptor complex but which results in the initiation of the biological response. This would be consistent with the antagonistic action of des-histidine-glucagon.

Insulin and glucagon

Our findings on the structure of glucagon contrast strongly with those on the structure of insulin, which is also a polypeptide hormone synthesised in the islets of Langerhans, but which

decreases, rather than increases, glucose levels in the blood. Glucagon is a flexible molecule which easily attains a helical structure in a hydrophobic environment. On the other hand, insulin has a well defined and relatively inflexible globular structure. In the latter case the importance of the three dimensional structure appears to result from the involvement of residues widely separated in the primary sequence in a relatively small receptor binding region¹¹. A stable tertiary structure, however, may have certain disadvantages; it will be degraded more slowly, and, therefore there may be less sophistication in the control of metabolism, as the effect of the hormone might linger on for some time. For insulin the degradation may be facilitated by the presence of a very exposed disulphide at A7-B7. The more flexible structure of glucagon would be more easily degraded by a proteolytic enzyme.

The two hormones, insulin and glucagon, do have similarities. Both associate in concentrated solutions through mainly hydrophobic interactions. The oligomers crystallise *in vitro* and a similar crystallisation seems to be involved in granulation of the hormones in storage. For instance, glucagon granules of certain species are crystalline rhombic dodecahedra (R. Lange and C. Klein, unpublished) and are identical in appearance to the crystals studied by X-ray analysis. Insulin granules are usually crystalline and often have a cubic or rhombohedral close packing of zinc insulin hexamers¹¹. Association of monomers in storage would undoubtedly decrease the rate of enzymatic proteolysis and increase the thermodynamic stability, in addition to providing a concentrated storage form¹¹. In both hormones, the hydrophobic residues which are involved in association also seem to be involved in receptor binding.

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Tertiary structural differences between microbial serine proteases and pancreatic serine enzymes

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Although primary structural homology between bacterial serine proteases and those from the mammalian pancreas is slight, two-thirds of the residues in the bacterial enzyme SGPB as seen at 2.8-Å resolution, adopt a similar polypeptide chain conformation to that of the chymotrypsin family. The three major regions of difference show how this family of proteolytic enzymes has developed from the more primitive bacterial to the relatively sophisticated pancreatic enzymes.

THE primary¹⁻⁸ and tertiary⁹⁻¹¹ structural homologies among the three pancreatic serine proteases have provided an excellent case for the common ancestral origin of these mammalian enzymes. There are also examples of serine proteases, from microbial sources, which have some regions of sequence identical to those of the pancreatic enzymes. The α -lytic protease (ref. 12 and refs. therein) from *Myxobacter* 495 and the two enzymes SGPA (ref. 13) and SGPB (ref. 14) from the extracellular culture filtrate (Pronase) of *Streptomyces griseus* are examples of enzymes with the active site sequences Gly-Asp-Ser-Gly-Gly and His-Cys identical to the corresponding sequences in the mammalian enzymes. These enzymes also have an aspartic acid at a position corresponding to the catalytically important Asp-102 of α -chymotrypsin⁹, elastase¹⁰ and trypsin¹¹. The overall sequence homology between the three enzymes from microbial sources and the pancreatic enzymes is, however, so low (< 20% identity, see Table 1) that tertiary structural homology can validly be questioned.

The question of a common ancestral origin of the bacterial and mammalian enzymes cannot be resolved without a determination of the tertiary structure of one of the microbial proteases. To support an argument for the divergent evolution of the Asp-Ser-Gly proteases, McLachlan and Shotton¹⁵ attempted to predict the tertiary structure of α -lytic protease from a knowledge of the polypeptide backbone conformations of α -chymotrypsin and elastase. Even though large deletions and insertions were required to align the two sequences, these authors were able to fit the amino acid sequence of α -lytic protease into the main-chain backbone of elastase with relatively few contraventions of conventional wisdom concerning globular protein conformation. The results of that study gave promise that the tertiary structures of homologous and functionally similar protein molecules could be predicted from a knowledge of the tertiary structure of one member.

Subtilisin BPN' has an active site geometry very similar to that of the pancreatic serine protease¹⁶. There is, however, no primary or tertiary structural homology of this bacterial enzyme with the mammalian enzymes. Thus for these two

protease classes this particular active site has been achieved through convergent lines of evolution.

To establish the relationship of the bacterial serine proteases with those from the mammalian pancreas we have determined the structure of SGPB at 2.8-Å resolution by X-ray crystallography. Some of the conclusions reached by McLachlan and Shotton¹⁵ are borne out by the present analysis; however, three important regions of the microbial enzymes have no tertiary-structural counterpart in the mammalian pancreatic enzymes.

Experimental procedure

Suitable single crystals of SGPB are obtained from 0.7–1.2 M KH_2PO_4 solutions at pH 4.2. These crystals have the symmetry of space group $P2_12_12$ and unit cell dimensions of $a = 44.16$, $b = 108.91$ and $c = 37.34$ Å. Details of the crystal growth, data collection and processing were reported in a preliminary publication on the 4.5-Å resolution structure of SGPB (ref. 17). In the study reported here Ni-filtered $\text{CuK}\alpha$ radiation from a tube operated at 40 kV and 16 MA was used. This reduction in incident beam intensity from conditions reported earlier¹⁷ increased the lifetime of the crystals by a factor of 3.5 and allowed the measurement of the complete 2.8-Å sphere of data (h kl and Friedel mate h kl , 9,500 reflections) from a single crystal of the native and each isomorphous derivative. The maximum decrease in intensity of the standard reflections was about 12% during the 120 h required to collect these data.

The heavy atom derivatives used in our analysis were prepared by soaking the native enzyme crystals in 1.3 M KH_2PO_4 solutions containing the following heavy atom compounds: 5 mM $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ for 72 h; 2 mM mersalyl for 48 h; a double derivative from 5 mM $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ for 12 h first followed by a mixed solution of 5 mM $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ and 2 mM mersalyl for 24 h; 10 mM K_3IrCl_6 for 72 h and 10 mM K_3IrCl_6 for 24 h. The heavy atom binding sites for these five useful derivatives were identified from cross-phased Fourier maps using the preliminary 4.5-Å phases¹⁷.

A summary of the final heavy atom parameters is included in Table 2. The agreement indices for these five derivatives varied from $R_c = 0.494$ to $R_c = 0.575$, where R_c is the Cullis R factor defined by: $\sum ||F_{PH} - F_P| - |f_H|| / \sum |F_{PH} - F_P|$ summed over the centric data only. The variation of the ratio of heavy atom scattering (f_H) to the lack of closure error (E_H) as a function of $\sin^2\theta/\lambda^2$ and the mean figures of merit for these several ranges are drawn in Fig. 1. All these criteria are well within accepted

Table 1 Amino acid identity matrix for the five proteins in Fig. 2

	SGPA	SGPB	α -Lytic	Chymo A	Elastase
SGPA	—	59.0%	35.3%	17.9%	17.9%
SGPB		—	36.0%	17.2%	20.4%
α -Lytic			—	17.7%	19.2%
Chymo A				—	38.8%
Elastase					—

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limits for a good determination of the protein phases by the multiple isomorphous replacement method.

Sites 4 and 2 (Table 2) for the $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ derivative were interpreted from a double difference map as a movement of the sulphur side chain of Met-162 on binding the platinum. Site 2 of the 24 h soaked K_3IrCl_6 derivative was interpreted as the loss of one of the bound phosphate groups in the region of the active site Ser-195 (see below). All the heavy atom sites in Table 2 behaved satisfactorily during the least squares refinement cycles.

The electron density map was computed with the native structure factor amplitudes and the best phases¹⁸ derived from the final phasing cycle. Interpretation of this map led to the structure on which the following discussion is based. The phasing program was a locally adapted version of one written by Dr M. G. Rossman; other programs were derived and adapted from the X-ray 70 system compiled by Dr J. Stewart.

Description of structure

To facilitate the comparison of the tertiary structures of the microbial serine proteases with those of the pancreatic enzymes, we have included in Fig. 2 the sequences of SGPB (ref. 14), SGPA (ref. 13), α -lytic protease¹², bovine chymotrypsinogen A (ref. 19) and porcine elastase⁸. The numbering scheme is that of bovine chymotrypsinogen A and chemically similar residues are enclosed in solid lines. The alignment presented here differs in some respects from the alignments previously published¹³⁻¹⁵ and has been based on tertiary structural similarities, a procedure strongly urged by Dickerson²⁰. Figure 2 and Table 2 show that there is considerably more sequence homology among the three microbial serine proteases than between the microbial and pancreatic protease groups.

The amino acid sequence data on SGPB (ref. 14) was invaluable for the interpretation of the 2.8-Å electron density map. The published tentative sequence had to be altered to accommodate two extra residues at positions where the sequence data were incomplete. At residue 65A the sequence Thr-Trp-Ala was altered to Thr-Trp-Trp-Ala to account for an extra lobe of density in the internal hydrophobic core. Chemical and spectrophotometric determinations of Trp in SGPB are also consistent with two tryptophan residues. (L. Jurásek and G. Willick, personal communication). The second region was at residue 186L (Asp-Val-Tyr) which was more consistent with a sequence Asp-Val-Val-Tyr for the interpretation of the

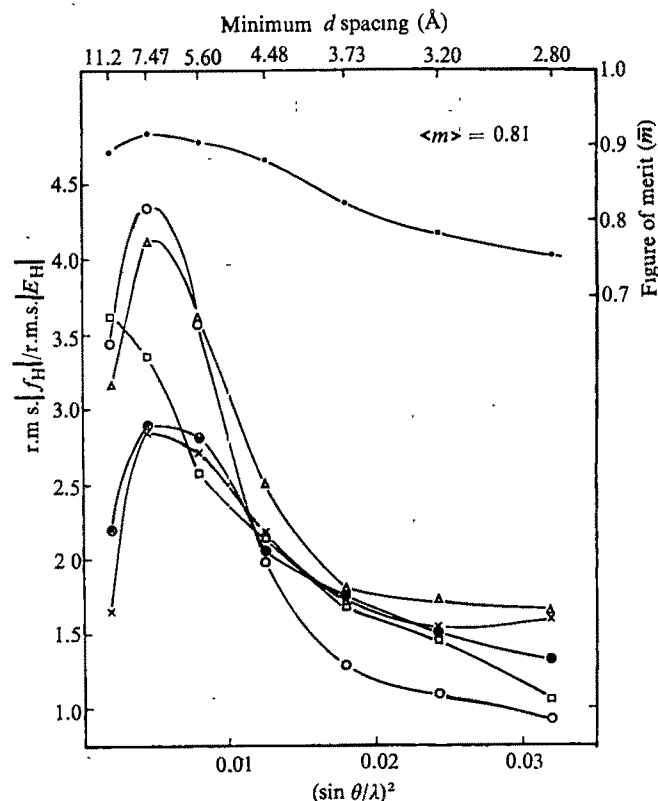


Fig. 1 Variation of the ratio of r.m.s. $|f_H|$ to r.m.s. $|E_H|$ and the figure of merit (\bar{m}) as functions of $\sin^2\theta/\lambda^2$. The r.m.s. $|f_H|$ is the root mean square heavy atom structure factor amplitude, r.m.s. $|E_H|$ is the root mean square lack of closure of the phase triangles. The derivatives are represented by the following symbols: \circ , $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$; \times , mersalyl; Δ , double derivative; \bullet , 3-d soaked K_3IrCl_6 ; \square , 1-d soaked K_3IrCl_6 .

electron density. The extra valine is consistent with the amino acid analyses of peptides derived from this unsequenced region because of the resistance of a valylvaline peptide bond to acid hydrolysis. There are thus 186 amino acid residues in the SGPB molecule.

Several sections of the 2.8-Å resolution electron density map computed over the region of the active site are shown in Fig. 3.

Table 2 Refined heavy atom parameters 2.8-Å data

Derivative	Site	x/a	y/b	z/c	A^*	B^\dagger
$\text{Pt}(\text{NH}_3)_2\text{Cl}_2$	1	0.8198	0.3490	0.7848	92.7	78.4
	2	0.835	0.3345	0.683	12.3	40.9
	3	0.821	0.3648	0.723	28.0	56.4
	4	0.804	0.3515	0.835	-17.6	5.6
Mersalyl‡	1	0.6823	0.4886	0.0317	18.8	0.6
	2	0.3422	0.3624	0.2312	29.7	48.6
	3	0.469	0.408	0.447	4.6	30.1
	4	0.8205	0.3500	0.7841	61.0	70.3
$\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ + mersalyl	2	0.839	0.337	0.683	4.2	7.2
	3	0.821	0.366	0.715	19.8	94.3
	4	0.807	0.351	0.829	-17.0	23.2
	5	0.6820	0.4884	0.0305	24.1	5.0
	6	0.3432	0.3628	0.2286	26.7	25.9
	7	0.468	0.409	0.436	10.3	67.5
	8	0.825	0.321	0.719	7.9	66.3
	9	0.896	0.356	0.747	6.0	35.1
	10	0.293	0.326	0.273	2.6	1.0
	11	0.5	0.5	0.8085	16.4	17.6
K_3IrCl_6 (3-d soak)	1	0.5	0.5	0.8065	25.7	24.5
K_3IrCl_6 (1-d soak)	2	0.4658	0.3903	0.2342	-3.7	59.4

* A is the site occupancy on a relative scale. A negative value of A corresponds to a site occupied in the native protein but not in the derivative.

† B is the isotropic temperature factor coefficient, \AA^2 .

‡ Mersalyl, o -($\text{NaO}_2\text{CCH}_2\text{O}$) C_6H_4 ($\text{CONHCH}_2\text{CH}(\text{OCH}_3)\text{CH}_2\text{HgOH}$).

	15A	15B	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	36A	36B	36C	37	38	39			
SGPB	---	---	Ile	Ser	Gly	Gly	---	---	---	---	---	---	---	---	---	Asp	Ala	Ile	Tyr	Ser	Ser	---	---	---	---	---	---	---	Thr			
SGPA	---	---	Ile	Ala	Gly	Gly	---	---	---	---	---	---	---	---	---	Glu	Ala	Ile	Thr	Thr	Gly	---	---	---	---	---	---	---	Gly			
α-LP	Ala	Asn	Ile	Val	Gly	Gly	---	---	---	---	---	---	---	---	---	Ile	Glu	Tyr	Ser	Ile	Asn	Asn	---	---	---	---	---	---	Ala			
Chymo A	---	---	Ile	Val	Asn	Gly	Glu	Glu	Ala	Val	Pro	Gly	Ser	Trp	Pro	Trp	Gln	Val	Ser	Leu	Gln	Asp	Lys	---	---	---	---	Thr	Gly	Phe		
Elastase	---	---	Val	Val	Gly	Gly	Thr	Glu	Ala	Gln	Arg	Asn	Ser	Trp	Pro	Ser	Gln	Ile	Ser	Leu	Gln	Tyr	Arg	Ser	Gly	Ser	Ser	Trp	Ala			
	40	41	42	43	44	45	46	47	48	48A	48B	48C	48D	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64			
SGPB	Gly	Arg	Cys	Ser	Leu	Gly	Phe	Asn	Val	Arg	Ser	Gly	Ser	Thr	Tyr	Tyr	Phe	Leu	Thr	Ala	Gly	His	Cys	Thr	Asp	---	Gly	Ala	Thr			
SGPA	Ser	Arg	Cys	Ser	Leu	Gly	Phe	Asn	Val	Ser	Val	Asn	Gly	Val	Ala	His	Ala	Leu	Thr	Ala	Gly	His	Cys	Thr	Ser	---	Asn	Ile	Ser			
α-LP	Ser	Leu	Cys	Ser	Val	Gly	Phe	Ser	Val	Thr	Arg	Gly	Ala	Thr	Lys	Gly	Phe	Val	Thr	Ala	Gly	His	Cys	Gly	Thr	Val	Asn	Ala	Thr			
Chymo A	His	Phe	Cys	Gly	Gly	Ser	Leu	Ile	Asn	---	---	---	---	---	Glu	Asn	Trp	Val	Val	Thr	Ala	Ala	His	Cys	Gly	Val	Thr	Thr	Ser	Asp		
Elastase	His	Thr	Cys	Gly	Gly	Thr	Leu	Ile	Arg	---	---	---	---	---	Gln	Asn	Trp	Val	Met	Thr	Ala	Ala	His	Cys	Val	Asp	Arg	Glu	Leu	Thr		
	65	65A	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92			
SGPB	Gly	Thr	Trp	Trp	Ala	Asn	Ser	Ala	---	---	---	---	---	---	---	Arg	Thr	Thr	Val	Leu	Gly	Thr	Thr	Ser	---	---	---	Gly	Ser			
SGPA	Ala	Ser	Trp	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	Ser	Ile	Gly	Thr	Arg	Thr	Gly	Thr			
α-LP	Ala	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	Arg	Ile	Gly	Gly	Ala	Val	Val	Gly			
Chymo A	Val	---	Val	Val	Ala	Gly	Glu	Phe	Asp	Gln	Gly	Ser	Ser	Ser	Glu	Lys	Ile	Gln	Lys	Leu	Lys	Ile	Ala	Lys	Val	Phe	Lys	Asn	Ser			
Elastase	Phe	Arg	Val	Val	Val	Gly	Glu	His	Asn	Leu	Asn	Gln	Asn	Asn	Gly	Thr	Glu	Gln	Tyr	Val	Gly	Val	Gln	Lys	Ile	Val	Val	His	Pro			
	93	94	95	96	97	98	99	99A	99B	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119			
SGPB	Ser	Phe	---	---	---	---	---	Pro	---	Asn	Asn	Asp	Tyr	Gly	Ile	Val	Arg	Tyr	Thr	Asn	Thr	Thr	---	---	---	---	---	---				
SGPA	Ser	Phe	---	---	---	---	---	Pro	---	Asn	Asn	Asp	Tyr	Gly	Ile	Ile	Arg	His	Ser	Asn	Pro	Ala	---	---	---	---	---	---				
α-LP	Thr	Phe	Ala	Ala	Arg	Val	Phe	Pro	---	Gly	Asn	Asp	Arg	Ala	Trp	Val	Ser	Leu	Thr	Ser	Ala	Gln	---	---	---	---	---	---				
Chymo A	Lys	Tyr	Asn	Ser	Leu	Thr	Ile	---	---	Asn	Asn	Asp	Ile	Thr	Leu	Leu	Lys	Leu	Ser	Thr	Ala	Ala	Ser	Phe	Ser	Gln	Thr	Val	Ser			
Elastase	Tyr	Trp	Asn	Thr	Asp	Asp	Val	Ala	Ala	Gly	Tyr	Asp	Ile	Ala	Leu	Leu	Arg	Leu	Ala	Gln	Ser	Val	Thr	Leu	Asn	Ser	Tyr	Val	Gln			
	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148			
SGPB	---	---	---	Ile	Pro	Lys	Asp	Gly	Thr	Val	Gly	Gly	---	---	---	---	Gln	Asp	Ile	Thr	Ser	---	---	---	---	---	---	---				
SGPA	---	Ala	Ala	Asn	Gly	Arg	Val	Tyr	Leu	Tyr	Asn	Gly	---	---	---	---	Ser	Tyr	Gln	Asp	Ile	Thr	Thr	---	---	---	---	---				
α-LP	---	Thr	Leu	Leu	Pro	Arg	Val	Ala	Asn	Gly	Ser	---	Ser	---	---	---	Phe	Val	Thr	Val	Arg	Gly	---	---	---	---	---	---				
Chymo A	Ala	Val	Cys	Leu	Pro	Ser	Ala	Ser	Asp	Asp	Phe	Ala	Ala	Gly	Thr	Thr	Cys	Val	Thr	Thr	Gly	Trp	Gly	Leu	Thr	Arg	Tyr	Thr	Asn			
Elastase	Leu	Gly	Val	Leu	Pro	Arg	Ala	Gly	Thr	Ile	Leu	Ala	Asn	Asn	Ser	Pro	Cys	Tyr	Ile	Thr	Gly	Trp	Gly	Leu	Thr	Arg	---	Thr	Asn			
	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	170	171	172	173	174	175				
SGPB	---	---	---	---	---	---	Ala	Ala	Asn	Ala	Thr	Val	Gly	Met	Ala	---	---	---	Val	Thr	Arg	Arg	---	---	---	---	---	Gly				
SGPA	---	---	---	---	---	---	Ala	Gly	Asn	Ala	Phe	Val	Gly	Gln	Ala	---	---	---	Val	Gln	Arg	---	---	---	---	---	---	Ser	Gly			
α-LP	---	---	---	---	---	---	Ser	Thr	Glu	Ala	Ala	Val	Gly	Ala	Ala	---	---	---	Val	Cys	Arg	---	---	---	---	---	---	Ser	Gly			
Chymo A	Ala	Asn	Thr	Pro	Asp	Arg	Leu	Gln	Gln	Ala	Ser	Leu	Pro	Leu	Leu	Ser	Asn	Thr	Asn	Cys	Lys	Lys	---	---	---	---	Tyr	Trp	Gly	Thr	Lys	
ELASTASE	Gly	Gln	Leu	Ala	Gln	Thr	Leu	Gln	Gln	Ala	Tyr	Leu	Pro	Thr	Val	Asp	Tyr	Ala	Ile	Cys	Ser	Ser	Ser	Ser	Tyr	Trp	Gly	Ser	Thr			
	176	177	178	179	180	181	182	183	184	185	186	186	186	186	186	186	186	186	186	186	186	186	186	186	186	186	186	186	186			
SGPB	Ser	Thr	Thr	Gly	Thr	His	Ser	Gly	Ser	Val	Thr	Ala	Leu	Asn	Ala	Thr	Val	Asn	Tyr	Gly	Gly	Gly	Asp	Val	Val	Tyr	Gly	Met	Ile			
SGPA	Ser	Thr	Thr	Gly	Leu	Arg	Ser	Gly	Ser	Val	Thr	Gly	Leu	Asn	Ala	Thr	Val	Asn	Tyr	Gly	Ser	Ser	Gly	Ile	Val	Tyr	Gly	Met	Ile			
α-LP	Arg	Thr	Thr	Gly	Tyr	Gln	Cys	Gly	Thr	Ile	Thr	Ala	Lys	Asn	Val	Thr	Ala	Asn	Tyr	Ala	---	Glu	Gly	Ala	Val	Arg	Gly	Leu	Thr			
Chymo A	Ile	Lys	Asp	Ala	Met	Ile	Cys	Ala	Gly	Ala	Ser	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
Elastase	Val	Lys	Asn	Ser	Met	Val	Cys	Ala	Gly	Gly	Asn	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
	187	188	188	189	190	191	192	192	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212			
SGPB	Arg	Thr	---	Asn	Val	Cys	Ala	Glu	Pro	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Tyr	Ser	Gly	Thr	---	---	---	---	---	---	---	Arg	Ala	Ile	Gly	Leu
SGPA	Gln	Thr	---	Asn	Val	Cys	Ala	Gln	Pro	Gly	Asp	Ser	Gly	Gly	Ser	Leu	Phe	Ala	Gly	Ser	---	---	---	---	---	---	---	Thr	Ala	Leu	Gly	Leu
α-LP	Gln	Gly	---	Asn	Ala	Cys	Met	Gly	Arg	Gly	Asp	Ser	Gly	Gly	Ser	Trp	Ile	Thr	Ser	Ala	Gly	---	---	---	---	---	---	Gln	Ala	Gln	Gly	Val
Chymo A	Gly	Val	---	Ser	Ser	Cys	Met	---	---	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Val	Cys	Lys	Lys	Asn	Gly	Ala	Trp	Thr	Leu	Val	Gly	Ile	---	---	
Elastase	Gly	Val	Arg	Ser	Gly	Cys	Gln	---	---	Gly	Asp	Ser	Gly	Gly	Pro	Leu	His	Cys	Leu	Val	Asn	Gly	Gln	Tyr	Ala	Val	His	Gly	Val	---	---	

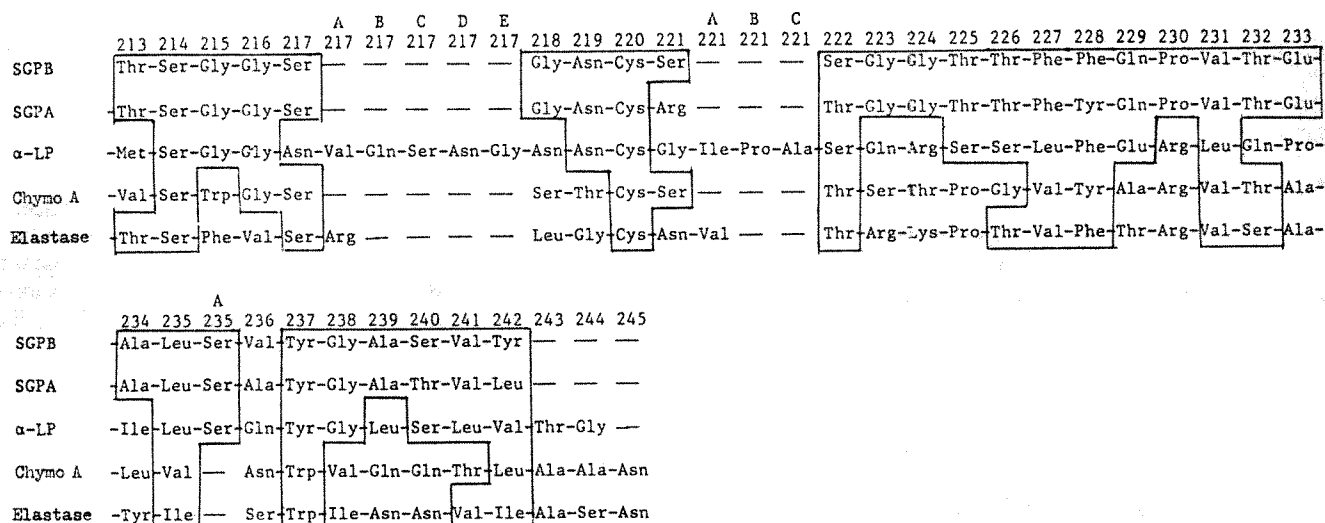


Fig. 2 Comparison of the amino acid sequences of SGPB, SGPA, α -lytic protease, bovine chymotrypsinogen A and porcine elastase. The sequence numbering is that of chymotrypsinogen A (ref. 19). Sequence homologies (chemically similar residues) are denoted by enclosing the amino acid residues within solid lines.

The disposition of the important residues of the catalytic triad is the same as that found for the other pancreatic serine proteases⁹⁻¹¹. Asp-102 is an internal residue and makes a close contact with His-57. The β -hydroxyl group of Ser-195 is also close to His-57, however we feel that the conformation in this region is different in minor respects to that in the active enzyme. The pH optimum is 7.0–8.0 for SGPB and as our study was done at pH 4.2 the enzyme is inactive. There is electron density close to the β -hydroxyl group of Ser-195 which we have tentatively assigned to two phosphate ions that interact with Ser-195 and a segment of the main chain between residues 216 and 217. There is a sulphate binding site observed at low pH in the active site region of one of the molecules of α -chymotrypsin^{21,22} and similar to the present phosphate binding site in SGPB. There is also a sulphate anion bound to the active site in elastase at pH 5.0 and this is replaced by two water molecules as the pH is raised to 8.5 (ref. 23). Serine protease inactivation at low pH may well derive in part from this anion binding in the active site region.

Polypeptide chain conformation

Figure 4 shows in diagrammatic fashion the path of the polypeptide chain. This view corresponds approximately to the standard view of the pancreatic enzymes depicting the molecule from a vantage point over the active site region. The polypeptide chain is folded to produce two hydrophobic cores with the active site located at their junction in a manner similar to that found in the pancreatic proteases. The first core which includes residues His-57 and Asp-102 is made up by folding the first 80 residues (16–128) in an extended conformation producing four major loops. The path taken by the chain in this first section of the molecule is similar to the conformation of the main chain in the mammalian proteases, with some notable exceptions.

The amino-terminal residue Ile-16 is not involved in an internal ion-pair interaction with Asp-194. Figure 4 shows that the amino terminus is on the surface of the enzyme with the *sec*-butyl side chain contributing to the first hydrophobic core and the amino group involved in close contact (possibly ion-pair interaction) with the β -carboxyl group of Asp-126. The chain length from the amino terminus to the 1/2 Cys-42 is much shorter in the microbial enzymes than in the pancreatic enzymes (Fig. 2). McLachlan and Shotton¹⁵ pointed out that

this short chain length was not consistent with the conformation of the polypeptide backbone in this region being identical in the two enzyme families. They did, however, suggest that with a minor conformational change the N-terminal amino group of α -lytic protease could form an ion pair with Asp-194. That this is not the case in the microbial enzymes is indicated by the present model of SGPB and was suggested earlier by the chemical work of Kaplan and Dugas²⁴, who showed that N-acetylation of the α -amino group in α -lytic protease had no effect on its enzymatic activity.

From the 1/2 Cys-42 the main chain traverses the molecule forming the so-called histidine loop before returning to complete the disulphide bridge at 1/2 Cys-58. This segment of the chain has four additional residues, 48A–48D, which are accommodated at the distal end of the histidine loop. Each of the three microbial enzymes SGPA, SGPB and α -lytic protease has these four extra residues.

Figure 2 shows that between residues 65 and 84 a large segment of the main chain of the pancreatic proteases is missing in SGPA and α -lytic protease, whereas only seven residues are deleted in SGPB. This region corresponds to the uranyl loop of α -chymotrypsin²⁵. In SGPA and α -lytic protease this loop is most certainly missing; however, Figure 4 shows that SGPB has a loop that corresponds to the uranyl loop of the pancreatic proteases albeit seven residues shorter.

The aspartate loop, so named because it contains the catalytically important Asp-102, makes up the fourth major loop in the first half of the molecule. The chain in SGPA and SGPB is five amino acids shorter than the chains of the mammalian enzymes and α -lytic protease. The important aromatic side chain (residue 94 which is Phe in the three microbial enzymes) is, however, present and in the SGPB structure serves partially to protect the Asp-102, His-57 interaction from exposure to the solvent. Those residues that are not present in SGPA and SGPB (95–99 inclusive) would form the distal end of the aspartate loop which also serves to shield the His-57, Asp-102 interaction in the mammalian enzymes.

Figure 2 shows that many deletions and insertions are required to produce some semblance of sequence homology between the mammalian enzymes and the microbial proteases in the region 112–191. Olsen *et al.*¹² noted that this region of α -lytic protease shows little homology with the pancreatic enzymes and that the sequence alignment for this section has been rather arbitrary.

Indeed in the microbial enzymes the autolysis loop is missing, but the polypeptide chain follows a path different from that taken in the mammalian proteases (Fig. 4). At residue 125, the only lysine residue of SGPB, the ϵ -NH₃⁺ group makes an ion pair with the α -carboxyl group of the terminal tyrosine-242. It is interesting that both the C terminus and the N terminus of SGPB are involved in ion-pair interactions with the neighbouring residues Lys-125 and Asp-126, respectively. Both SGPA and α -lytic protease have only the basic residue (Arg) at position 125 which may have some influence on the relative stabilities of SGPA and SGPB to denaturing conditions²⁶, although the unusual stability of SGPB to denaturants should be investigated more fully.

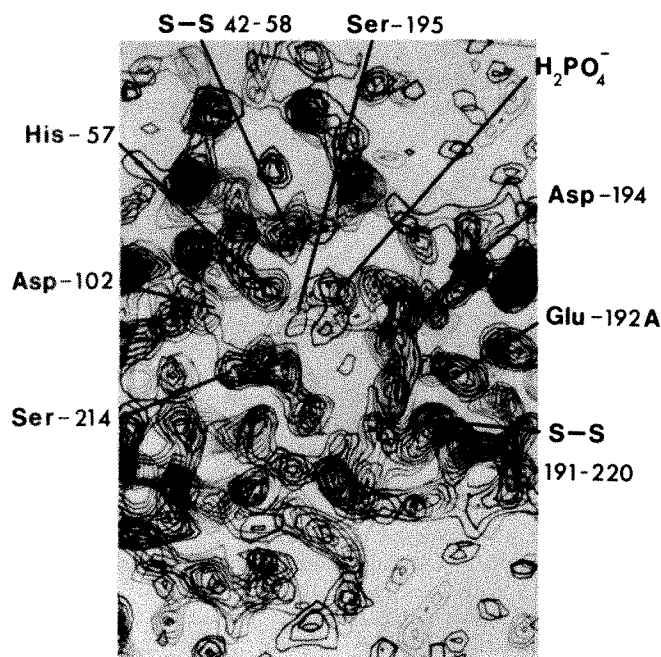


Fig. 3 Six sections (1-Å spacing) of the 2.8-Å electron density map of the SGPB molecule in the region of the active site. The residues that make up the active site are labelled. The two disulphide bridges 42-58 and 191-220 are also designated on these sections. The view of the molecule in these sections corresponds approximately to that shown in Fig. 4.

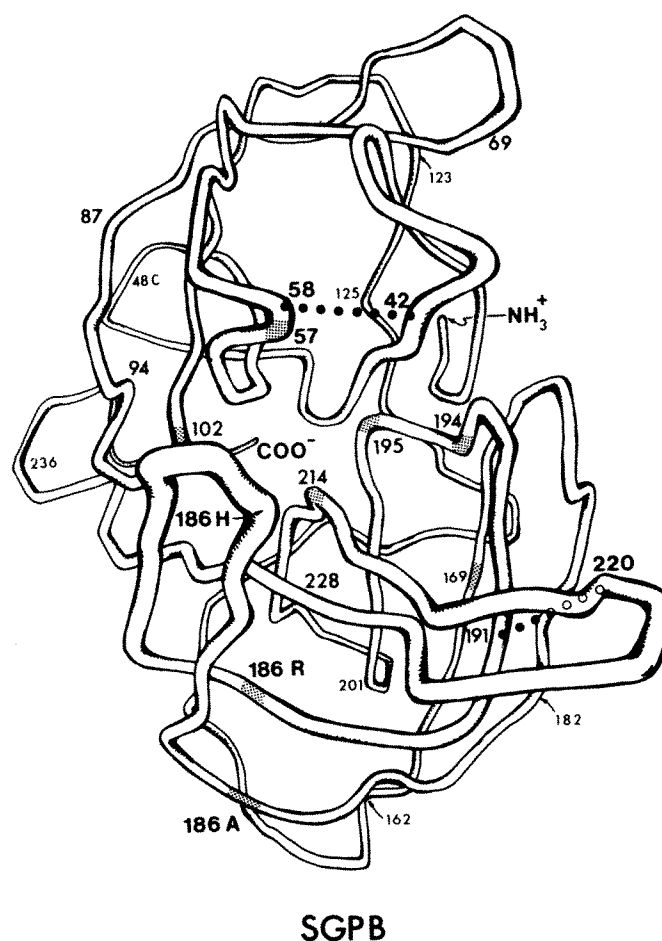
Two other segments of the polypeptide chain in SGPB in the region 123-191 are profoundly different in their locations relative to the tertiary structures of the pancreatic enzymes. The first is that segment of chain that is analogous to the methionine loop in the pancreatic enzymes (residues 168-182). The conformation of the methionine loop in SGPB is such that it occupies a volume in the SGPB molecule that corresponds to the autolysis loop of the pancreatic enzymes. This important structural difference places the side chain of a basic residue, Arg-169, in the appropriate orientation for the internally buried, positively charged guanidinium group to make an internal salt bridge with the carboxylate of Asp-194 (Fig. 4). In view of the methionine loop conformation observed for SGPB it seems unlikely that the microbial enzymes have a zymogen precursor. The enzymes must be catalytically active immediately the folding process has been completed after their synthesis. It could have been predicted from the results of Kaplan and Dugas²⁴ that the positive charge in this crucial ion pair must originate from some other portion of the molecule, but the residue providing the positive charge would have been impossible to predict with confidence. Even though the primary function of this basic residue at position 169 has been lost

through the evolution of the pancreatic serine proteases the basic nature of this residue is conserved in all serine proteases except elastase²⁷.

The other major difference in polypeptide chain conformation occurs at position 186 in the sequence of the microbial enzymes where there are 18 additional residues in SGPA and SGPB, and 17 in α -lytic protease. Clearly these residues do not have a counterpart in the pancreatic proteases. Therefore McLachlan and Shotton postulated that they formed an external loop (termed the compensating extra loop) which would substitute for the residues in the missing autolysis loop. As Fig. 4 shows, residues 186A-186R are not involved with providing a hydrophobic pocket that serves to bury the amino terminus, but rather they are in an extended β loop located on the opposite side of the molecule near the aspartate loop and this extra loop functions as a shield for the Asp-102, His-57 interaction from solvent. The five residues that are missing in the aspartate loop of SGPA and SGPB occur at a point where the loop bends back on itself in an antiparallel β -sheet conformation. These extra residues (95-99) in the pancreatic serine proteases occupy a volume that also shields the Asp-102, His-57 interaction from solvent (namely the hydrophobic residues at position 99). Therefore it appears that the microbial enzymes accomplish this shielding function in a rather extravagant fashion using an extra loop of 17 or 18 amino acid residues to do the task that five residues perform for the pancreatic serine proteases.

From the second disulphide bridge 1/2 Cys-191 to 1/2 Cys-220 the conformation of the polypeptide chain in SGPB

Fig. 4 A diagrammatic representation of the backbone conformation of the polypeptide chain from the 2.8-Å electron density map of SGPB. The labelled regions refer to the residue numbers of Fig. 2. The stippled segments correspond to approximate α -carbon positions for the designated residues. The disulphide bridges 42-58 and 191-220 are indicated.



closely resembles that in the pancreatic family. The residues involved in forming the specificity depression as well as the residues forming the serine loop are in this latter segment of the molecule. To form the serine loop the polypeptide chain traverses the central portion of the molecule and bends back on itself in a manner similar to the histidine loop conformation. From residue 214 to residue 228 there is a large loop of anti-parallel β -pleated sheet structure before the chain emerges from under the extra loop and completes the only two turns of α helix in the whole molecule, residues 231–238. This conformation of the chain is directly homologous to the main chain folding in the pancreatic enzymes. In SGPB, however, the final four amino acids are in an extended conformation and the ultimate residue, Tyr-242, has its aromatic side chain tucked back up into the hydrophobic region between the two central cores.

Active site and primary specificity site

The folding of the polypeptide chain of SGPB produces an active site region between the two hydrophobic cores in the enzyme that qualitatively resembles those active site regions in the pancreatic serine proteases. The amino acid side chains that are involved in the catalytic mechanism, that is, Asp-102, His-57, Ser-195 and Asp-194 are all similarly disposed in these two enzyme families and the catalytic triad is conserved as expected. A second serine residue, Ser-214, occupies a position that places its β -hydroxyl group within hydrogen bond distance of Asp-102 (Fig. 3). The importance of this second serine at position 214 has been pointed out by Kraut *et al.*²⁸ and its functional role in the catalytic mechanism is further substantiated by the study reported here. A much greater degree of homology than shown by McLachlan and Shotton¹⁵ is attained by having a three-residue deletion at position 205 in the α -lytic sequence as indicated in Fig. 2, which correctly places a serine residue at position 214.

The primary binding site of a pipsyl (*p*-iodobenzene sulphonyl) inhibitor on the SGPB molecule was described in the 4.5-Å resolution structural study¹⁷. The present 2.8-Å resolution map shows that this site is made up primarily of two short segments of main chain, residues 192–192B and residues 215–218. There is no evidence for a hydrophobic binding pocket or tosyl hole as seen in the α -chymotrypsin structure^{25,29}, however, in SGPB the binding site is a depression on the enzyme surface. The broad specificity of SGPB³⁰ is consistent with this binding site. The fact that SGPB will also hydrolyse bonds on the carboxyl side of basic residues may be due to the involvement of Glu-192A in this binding. With the second segment of the binding site made up chiefly of glycine residues, the whole binding region involves principally polypeptide backbone and has a shape that would accommodate large hydrophobic side chains¹⁷.

The binding pocket of α -lytic protease deserves comment. The revised alignment presented in Fig. 2 and similar to that proposed by Johnson and Smillie¹³ inserts a pentapeptide 217A–217E in this region. The α -lytic enzyme has a specificity similar to that of elastase and differs from the α -chymotrypsin specificity in that it does not cleave polypeptide chains on the carboxyl side of aromatic side chains. Clearly the extra five residues present in α -lytic protease would alter this region of the specificity depression from that seen in the SGPB structure, thus explaining the different specificities of these two enzymes.

SGPA has a broader but essentially similar specificity to that of SGPB³¹. Cleavage of the insulin B chain by SGPA occurs on the carboxyl side of Leu, Tyr, Phe, Arg and to a lesser degree Glu and Val. This similar specificity to SGPB and the fact that the sequence homologies between SGPA and SGPB are so great in the region of the substrate binding site, indicate that these two enzymes have almost identical tertiary structures in this region.

Evolutionary inferences

Although there are large differences in chain length (186 amino acids in SGPB compared with ~240 for the pancreatic enzymes) and relatively little in the way of sequence homology (Table 1) the overall tertiary structures are similar. Those sections of polypeptide chain which are almost similar, the histidine loop, the aspartate loop, the serine loop and the specificity loop (214–227) provide the probable structural core of the serine proteases. This similarity of structure provides a strong case for the divergent evolution of these serine proteases and demonstrates that this class of enzyme is very ancient and had an ancestor common to the mammals and bacteria.

The tertiary structural differences between these enzymes from such phylogenetically distant organisms also provide information on the evolution of protein structure. The evolution of catalytically inactive zymogens by multicellular organisms is a result of the necessity for the organism to protect itself from auto-digestion. McLachlan³² has suggested a piecemeal growth mechanism for the insertion or deletion of single amino acids into surface loops of an already biologically active protein in such a way that the activity is conserved. This mechanism allows for the addition (or deletion) of surface loops and for the concomitant improvement (or alteration) in biological activity. The structural core is conserved in going from the bacterial to the pancreatic enzymes; however, new stretches of chain have been added (in particular the N terminus and aspartate loop are lengthened, and the autolysis loop added) and other loops have been deleted (the extra compensating loop is lost after the acquisition of additional residues in the aspartate loop) or the function altered or lost (methionine loop).

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Regulation of cell cycle stage-specific transcription of histone genes from chromatin by non-histone chromosomal proteins

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RNA transcripts from chromatin of S phase but not G₁ cells contain histone-specific sequences. Chromatin reconstituted with S phase non-histone chromosomal proteins transcribes histone messenger RNA sequences whereas chromatin reconstituted with G₁ non-histone proteins does not. These results suggest that transcription of histone genes is regulated during the cell cycle and that non-histone proteins have a key role in this regulation.

SEVERAL lines of evidence suggest that non-histone chromosomal proteins are important in the regulation of gene expression in general¹⁻⁷, and specifically in the control of transcription during the cell cycle^{8,9}. Variations in the composition and metabolism of non-histone chromosomal proteins throughout the cell cycle are consistent with a regulatory function for these macromolecules¹⁰⁻¹⁷. More direct evidence that non-histone chromosomal proteins are regulatory macromolecules within this context comes from a series of chromatin reconstitution studies which demonstrate that non-histone chromosomal proteins are responsible for the increased transcriptional activity of S phase compared with mitotic chromatin^{8,9}.

In HeLa cells the synthesis of histones is confined to the S phase of the cell cycle¹⁸⁻²¹, and *in vitro* translation data have suggested that the messenger RNAs (mRNAs) for these basic chromosomal polypeptides are associated with a specific class of polysomes only at this time¹⁹⁻²³. We have added adenylic acid residues to the 3'-OH termini of histone mRNAs, and using RNA-dependent DNA polymerase, synthesised a histone complementary DNA (cDNA)²⁴. Using this high resolution probe, we have demonstrated that, consistent with the *in vitro* translation data, histone mRNA is associated with polyribosomes only during the S phase²⁵. In the present studies the histone cDNA was used to demonstrate that transcription *in vitro* of histone mRNA sequences is restricted to the S phase of the cell cycle and that non-histone chromosomal proteins are responsible for regulating the transcription from chromatin of those regions of the genome which contain the information for the synthesis of histones.

Characterisation of histone complementary DNA

To detect the presence of histone-specific RNA sequences, a single-stranded DNA complementary to histone mRNAs was synthesised using RNA-dependent DNA polymerase as described previously²⁴. 7-12S RNA was isolated from the polysomes of S phase HeLa S₃ cells and poly(A)-containing RNAs were removed by oligo(dT)-cellulose chromatography. The remaining 7-12S RNAs directed the synthesis of all five classes of histones in a cell-free protein synthesising system derived from wheat germ. Poly(A) (an average of 35 AMP residues) was added to the 3'-OH termini of the histone mRNAs with an ATP-poly nucleotidyltransferase isolated from maize seedlings. The isolation and properties of this enzyme have been reported²⁶. The polyadenylated mRNAs were then transcribed with RNA-dependent DNA polymerase from

Rous sarcoma virus using dT₁₀ as a primer. The mean sedimentation coefficient of the cDNA in alkaline sucrose was 6.1S which corresponds to a size of approximately 400 nucleotides^{27,28}. The kinetics of hybridisation of the cDNA probe to histone mRNA isolated from polysomes of S phase HeLa S₃ cells were measured to assess the purity of the template RNA and cDNA (Fig. 1). The reaction proceeds with a C_0t_1 of 1.7×10^{-2} (see also double reciprocal plot³⁰ of the data, Fig. 1 inset). Since the molar concentration of the nucleotide sequences in solution determines the rate of hybridisation, comparison of the C_0t_1 of the histone mRNA to that of a kinetic standard such as globin mRNA (complexity of 1,200 bases³¹ and C_0t_1 in similar conditions of 3.8×10^{-3})³² yields a calculated sequence complexity of approximately 5,400 bases which is two times greater than expected for the total complexity of the five histone messages. This is, however, within the range of variation found for the rate of RNA-DNA hybridisation^{30,33-35}. When cDNA and histone mRNA are hybridised in the absence of formamide at 75 °C (data not shown), the C_0t_1 is 5×10^{-3} . From the C_0t_1 of globin mRNA in these

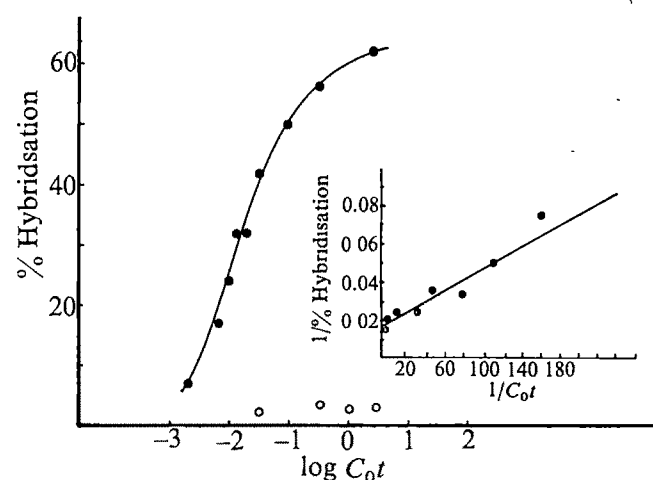


Fig. 1 Kinetics of annealing of histone cDNA to histone messenger RNA isolated from the polysomes of S phase HeLa S₃ cells. 400 c.p.m. of ³H-cDNA (27,000 d.p.m. ng⁻¹) were annealed at 52 °C in sealed glass capillary tubes in a volume of 15 μl containing 50% formamide, 0.5 M NaCl, 25 mM HEPES (pH 7.0) and 1 mM EDTA with either 0.03 or 0.19 μg of histone mRNA in the presence of 3.75 μg of *E. coli* RNA as carrier (●) or with 3.75 μg of *E. coli* RNA in identical conditions (○). The reaction mixtures were assayed for hybrid formation using fraction IV, single-strand specific S₁ nuclease isolated from *Aspergillus oryzae*²⁹. Each sample was incubated for 20 min in 2.0 ml of 30 mM sodium acetate, 0.3 M NaCl, 1 mM ZnSO₄, 5% glycerol (pH 4.6), containing S₁ nuclease at a concentration sufficient to degrade at least 95% of the single-stranded nucleic acids present. The amount of radioactive DNA resistant to digestion was determined by trichloroacetic acid precipitation. No background values have been subtracted. C_0t = mol ribonucleotides s l⁻¹. Inset: Double reciprocal plot of the kinetics of annealing of histone cDNA and histone mRNA. A background of 3% has been subtracted from each point.

conditions ($C_{ot}_1 = 2.0 \times 10^{-3}$)^{36,37} the sequence complexity of histone mRNA is estimated to be 3,000 bases. When the probe is annealed with *Escherichia coli* RNA in either of the above conditions, no significant level of hybrid formation is detected. The low level of S_1 nuclease resistant TCA-precipitable radioactivity may be accounted for by a limited amount (3%) of ^3H -cDNA which is not digested by the enzyme in the incubation conditions used. Thermal denaturation curves of the histone mRNA-cDNA hybrids exhibit a single transition with a T_m of 65 °C in 50% formamide-0.5 M NaCl-25 mM HEPES (pH 7.0)-1 mM EDTA and 95 °C in 0.5 M NaCl-25 mM HEPES (pH 7.0)-1 mM EDTA. These T_m values are consistent with the reported base composition of histone mRNA of 54% G-C (ref. 38).

Additional evidence for specificity of the histone cDNA is its ability to form hybrids with total polysomal RNA isolated from intact S phase HeLa cells ($C_{ot}_1 = 1.8$) and its lack of hybrid formation with G_1 polysomal RNA²⁵. From these findings it is reasonable to conclude that ribosomal (5S, 18S and 28S) and transfer RNA complementary sequences are not present in the histone cDNA. Furthermore, the polysomal RNA isolated from HeLa cells in which histone and DNA synthesis have been blocked by cytosine arabinoside does not form hybrids with the histone cDNA. These results are consistent with data from several laboratories which indicate that histone mRNA is not present on the polyribosomes of HeLa cells treated with inhibitors of DNA synthesis^{19,23,39-42} and additionally rule out the possibility that the cDNA contains detectable amounts of sequences complementary to other S-phase specific, non-polyadenylated RNAs which have been reported to be insensitive to cytosine arabinoside²³.

Transcription of histone-specific sequences from G_1 and S phase chromatin

Evidence has indicated that in HeLa cells histone synthesis is restricted to the S phase of the cell cycle and that the synthesis of these basic chromosomal polypeptides is dependent on concomitant DNA replication¹⁸⁻²⁰. It has also been shown that mRNAs for histones are associated with polysomes during S phase and not during other periods of the cell cycle^{19-21,23,25}. One might therefore anticipate that the synthesis of mRNAs for histones is restricted to the period of DNA replication. Direct evidence for this contention is lacking, however. Therefore, as an initial approach to studying histone gene transcription we examined the abilities of chromatin from G_1 and S phase cells to transcribe histone-specific sequences *in vitro*.

The degree to which *in vitro* RNA transcripts from G_1 and S phase chromatin hybridise to the histone ^3H -cDNA was determined. Figure 2 shows the polyacrylamide gel electrophoretic distribution of RNAs synthesised from HeLa S_3 cell chromatin with *E. coli* RNA polymerase. More than 90% of the RNAs synthesised migrated in the 4-14S region of the gels reflecting the synthesis of RNA molecules which are 75-1,500 nucleotides long. The data in Fig. 3 clearly indicate that although histone-specific sequences are present in the transcripts from S phase chromatin, such sequences are not detected in the transcripts from chromatin of G_1 phase cells. Specifically, the ^3H -cDNA hybridises to S phase chromatin transcripts with a C_{ot}_1 of 2.1×10^{-1} , whereas no hybridisation above the background level of 3% was detected with G_1 transcripts. The extent of hybridisation of transcripts from chromatin of exponentially growing HeLa cells ($C_{ot}_1 = 4 \times 10^{-1}$) was approximately 50% that observed for S phase transcripts (approximately 50% of an exponentially growing population of HeLa cells are undergoing DNA synthesis at any given time) suggesting that thymidine synchronisation used for obtaining S phase cells does not significantly alter transcription of histone mRNA sequences. It should be noted that the T_m (65 °C in 50% formamide-0.5 M NaCl-25 mM HEPES (pH 7.0)-1 mM EDTA and 95 °C in 0.5 M NaCl-25 mM HEPES (pH 7.0)-1 mM EDTA) of the hybrids formed between the ^3H -cDNA and S phase chromatin

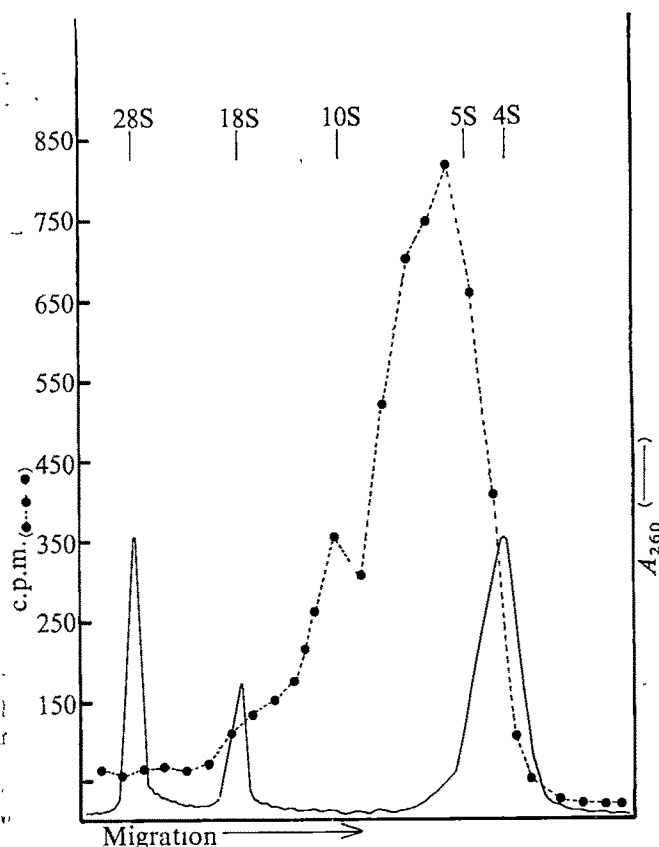


Fig. 2 Polyacrylamide gel electrophoresis of RNAs transcribed from HeLa S_3 cell chromatin in the presence of ^{14}C -ATP ($0.4 \mu\text{Ci ml}^{-1}$). Electrophoresis was carried out according to the method of Schochetman and Perry⁴³ in the presence of 4S, 18S and 28S RNAs as internal markers. RNA was transcribed using Fraction V *E. coli* RNA polymerase prepared according to the method of Berg *et al.*⁴⁴ Transcription was carried out for 70 min at 37 °C in a Dounce homogeniser fitted with a wide clearance pestle, and the reaction mixture was periodically homogenised to maintain chromatin solubility. The incubation mixture in a final volume of 10 ml contained: 0.04 M Tris (pH 8.0); 4 mM MgCl_2 ; 1 mM MnCl_2 ; 0.02 mM EDTA; 0.008% β -mercaptoethanol; 0.4 mM each of ATP, CTP, UTP, and GTP, $150 \mu\text{g ml}^{-1}$ of DNA as chromatin, and 600 U RNA polymerase. RNA was extracted as follows. The reaction was brought to a concentration of 1% SDS-0.1 M NaCl-0.01 M sodium acetate-1 mM EDTA (pH 5.4) and incubated at 37 °C for 15 min. Following two extractions with equal volumes of phenol and chloroform-isoamyl alcohol (24:1, v/v) and two extractions with chloroform-isoamyl alcohol, nucleic acids were precipitated with 3 volumes of ethanol. The pellet was resuspended in 10 mM Tris-0.1 M NaCl-5 mM MgCl_2 (pH 7.4) containing $40 \mu\text{g ml}^{-1}$ of DNase I and incubated at 37 °C for 60 min. Following one extraction with phenol-chloroform-isoamyl alcohol and two with chloroform-isoamyl alcohol, the aqueous phase containing the RNA transcripts was chromatographed on Sephadex G-50 fine and eluted with 50 mM Tris-0.1 M NaCl-1 mM EDTA (pH 7.2). RNA was precipitated with 3 volumes of ethanol. For hybridisation analysis the RNA was resuspended in 25 mM HEPES-0.5 M NaCl-1 mM EDTA, pH 7.0.

transcripts is identical to the T_m of the ^3H -cDNA-histone mRNA hybrids. From a comparison of the C_{ot}_1 values of the S phase chromatin transcript-cDNA and histone mRNA-cDNA hybridisation reactions it can be estimated that 8% of the S phase transcripts are histone-specific sequences. The figure of 8% is based on the assumption that the histone mRNA preparation consists entirely of histone mRNA sequences. The presence of other sequences in the preparation would require a corresponding reduction in this figure.

To rule out the possibility that endogenous RNAs associated with S phase chromatin at least in part account for the formation of hybrids with histone cDNA, the following approach was pursued. S phase chromatin was placed in the transcription mixture without RNA polymerase and an amount of *E. coli*

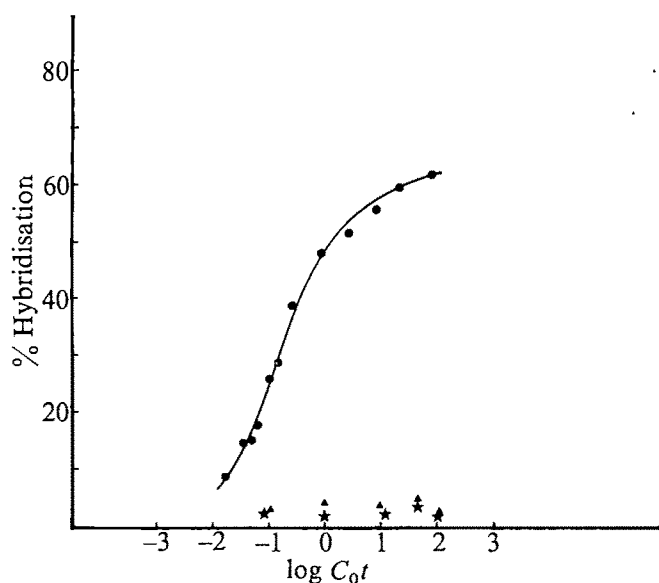


Fig. 3 Kinetics of annealing of histone cDNA to *in vitro* transcripts of chromatin from G_1 and S phase HeLa S_3 cells. 400 c.p.m. of ^3H -cDNA (27,000 d.p.m. ng^{-1}) were annealed at 52°C to either 0.15 or 1.5 μg of RNA transcripts from G_1 (\blacktriangle) or S phase (\bullet) chromatin. 400 c.p.m. of cDNA were also annealed to 1.5 μg of *E. coli* RNA isolated in the presence of S phase chromatin (\star). *E. coli* RNA was included in each reaction mixture so that the final amount of RNA was 3.75 μg . Exponentially growing HeLa S_3 cells were maintained in suspension culture in Joklik-modified Eagle's MEM supplemented with 3.5% each of calf and foetal calf serum. Cells were synchronised as described previously¹⁰. S phase cells were obtained by synchronisation with 2 cycles of 2 mM thymidine block. 3 h after release from the second thymidine block cells were collected, at this time 98% of the cells were in S phase as determined by autoradiographic assessment of ^3H -thymidine labelled nuclei. G_1 cells were obtained 3 h after selective detachment of mitotic cells from semiconfluent monolayers. 97% of the cells were in the G_1 phase of the cell cycle; incorporation of ^3H -thymidine into nuclei could not be detected autoradiographically, reflecting the complete absence of S phase cells. Nuclei were obtained by washing cells 4 times in 80 volumes of Earle's balanced salt solution and lysing the cells in 80 volumes of 80 mM NaCl–20 mM EDTA–1% Triton X-100 (pH 7.2). The nuclei were washed three times with the lysing medium and then twice with 0.15 M NaCl–0.01 M Tris (pH 8.0). Nuclei isolated in this manner are free of cytoplasmic material when examined by phase contrast and electron microscopy. Lysis of nuclei was achieved by suspending the nuclear pellet in triple glass-distilled water with several strokes of a wide-clearance Dounce homogeniser. The chromatin was allowed to swell at 4°C for 30 min, pelleted at 20,000g for 15 min, resuspended in distilled water, and again pelleted at 20,000g.

RNA equivalent to the amount of RNA transcribed from S phase chromatin was added. RNA was immediately extracted by the same procedure used for the isolation of *in vitro* transcripts. When this control RNA was annealed with the cDNA no significant level of hybridisation was observed (Fig. 3). Additionally, RNA isolated from S phase chromatin in the absence of carrier shows no hybrid formation with the cDNA. These results establish that endogenous, histone-specific sequences associated with S phase chromatin are not contributing significantly to the hybridisation observed with S phase *in vitro* transcripts. The findings presented so far are consistent with the argument that the genetic sequences which code for the synthesis of histones are available for transcription from chromatin during the S phase of the cell cycle but not during G_1 .

Regulation of histone-specific sequences by non-histone chromosomal proteins

Although evidence has been presented which strongly suggests that among the non-histone chromosomal proteins are macromolecules which are responsible for the regulation of tran-

scription during the cell cycle, the evidence is of a correlative nature. To examine directly the involvement of non-histone chromosomal proteins in the control of cell cycle stage-specific gene readout, we pursued the following approach. Chromatin isolated from G_1 and S phase cells was first dissociated, in 3 M NaCl–5 urea–0.01 M Tris (pH 8.3), and the DNA was pelleted at 150,000g for 36 h. Proteins were fractionated into histone and non-histone chromosomal protein fractions by the QAE–Sephadex method of Gilmour and Paul⁴⁵. Chromatin was reconstituted by the gradient dialysis procedure of Bekhor *et al.*⁴⁶. The details of these methods⁸ and evidence for fidelity of chromatin reconstitution^{46–48} have been reported. DNA was prepared by the method of Marmur⁴⁹, treated with pancreatic ribonuclease A (50 $\mu\text{g ml}^{-1}$ for 30 min at 37°C) and Pronase (50 $\mu\text{g ml}^{-1}$ for 2 h at 37°C), and then extracted twice with phenol and chloroform–isoamyl alcohol before use.

In vitro RNA transcripts from chromatin reconstituted with G_1 non-histone chromosomal proteins and from chromatin reconstituted with S phase non-histone chromosomal proteins were annealed with histone ^3H -cDNA. Figure 4 shows that RNA transcripts from chromatin reconstituted with S phase non-histone chromosomal proteins hybridise with histone cDNA although those from chromatin reconstituted with G_1 non-histone chromosomal proteins do not exhibit a significant degree of hybrid formation. It should be emphasised that the kinetics and extent of hybridisation with the cDNA are the same for transcripts of native S phase chromatin and transcripts of chromatin reconstituted with S phase non-histone chromosomal proteins (Fig. 4). Furthermore, the amounts of RNA transcribed and the recoveries during isolation of these transcripts from the native and reconstituted chromatin preparations are essentially identical. These results imply a functional role for non-histone chromosomal proteins in regulating the availability of histone sequences for transcription from chromatin and, taken together with the polysomal data presented previously²⁸, suggest that, in intact cells, regulation of histone gene expression during the cell cycle may reside, at least in part, at the transcriptional level. Such a regulatory role for non-histone chromosomal proteins is in agreement with results from several laboratories which have indicated that these

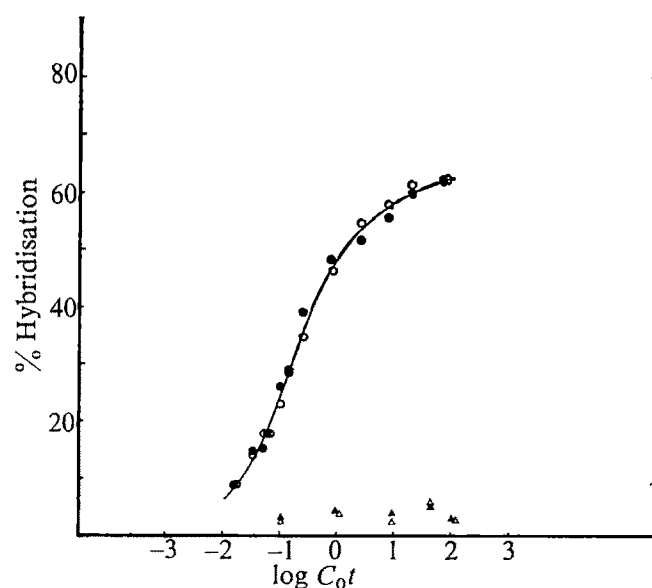


Fig. 4 Kinetics of annealing of histone cDNA to *in vitro* transcripts from native and reconstituted chromatin. 400 c.p.m. of ^3H -cDNA were annealed at 52°C with either 0.15 μg or 1.5 μg of RNA transcripts from chromatin reconstituted with S phase non-histone chromosomal proteins and DNA and histones pooled from G_1 and S phase cells (\circ), chromatin reconstituted with G_1 non-histone chromosomal proteins and DNA and histones pooled from G_1 and S phase cells (\triangle), native S phase chromatin (\bullet) and native G_1 chromatin (\blacktriangle). *E. coli* RNA was included in each reaction mixture so that the final amount of RNA was 3.75 μg .

proteins are responsible for the tissue-specific transcription of globin genes^{2,36,50}.

Although it seems that among the non-histone chromosomal proteins are macromolecules which regulate the transient readout of histone genes during the cell cycle, the regulatory elements involved have yet to be defined. It is not clear whether the non-histone chromosomal proteins which render histone sequences transcribable during S phase, are synthesised at the onset of DNA replication or whether they are pre-existing proteins which are enzymatically modified at this time. Post-translational modifications of non-histone chromosomal proteins, particularly phosphorylation, have been implicated as having a key role in dictating structural as well as transcriptional properties of the genome. Furthermore, it remains to be established whether control is of a positive or negative nature. Since the genetic sequences for histone mRNAs in HeLa cells are reiterated⁴², the possibility arises that a single cell may contain multiple copies of the proteins which control the expression of histone genes. This system may therefore be readily amenable to the isolation of such regulatory proteins.

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Theory and practice for studies of peptides by ¹⁵N nuclear magnetic resonance at natural abundance: gramicidin S

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The low-abundance isotope of nitrogen, ¹⁵N, is now accessible to study by the latest high resolution nuclear magnetic resonance techniques. Structure and motion in polypeptides of moderate size may now be usefully investigated in this way.

THE difficulties in observing magnetic resonance spectra (NMR) of the ¹⁵N nucleus at the natural abundance level (0.36%) are well established¹⁻³. Recent advances in technique⁴⁻⁶, however, specifically the pulse-Fourier transform method with ¹H noise decoupling, have made it possible to obtain useful spectra. The initial studies on natural abundance ¹⁵N NMR, however, used neat liquid samples^{4,7} or highly concentrated solutions of low molecular weight material^{3,5}. Of course the low sensitivity has occasionally been elevated for ¹⁵N (as for other nuclei, such as ¹³C) by isotopic enrichment⁸. Labelling techniques then can add a new dimension to mechanistic studies, as witnessed by the nitrosation work on peptide

models, in which the fate of ¹⁵N-labelled nitrite ion is followed by NMR⁹.

The rewards of studying ¹⁵N NMR are first, that one observes a high resolution nucleus that may participate directly in fundamental chemical and biological processes (for example, acid-base equilibria, tautomerism, hydrogen bonding and coordination), and second, that the spectra of complex nitrogen-containing molecules, for example peptides, should be far simpler, and therefore easier to interpret than their ¹³C- or ¹H-spectra which can be over-rich in resonances.

Some general considerations for ¹⁵N NMR

The sensitivity of ¹⁵N in an NMR experiment at constant field compared with ¹³C (both isotopes at natural abundance) is about 1:30. Thus, to obtain comparable signal-to-noise ratios from experiments using signal averaging on the two species at similar total nitrogen and carbon concentrations would take about 900 times longer for ¹⁵N than for ¹³C, which is already a difficult nucleus. A combination of larger sample volumes (25 mm outer diameter tubes) and a spectrometer

Table 1 ^{15}N Chemical shifts of some amino acid methyl ester hydrochlorides in water

$\begin{array}{c} \text{R} \\ \\ \text{H}_3\text{CO}_2\text{C}-\text{CH}-\text{NH}_3^+ \end{array}$			
R	$\delta^{15}\text{N}^*$	R	$\delta^{15}\text{N}^*$
H (Glycine)	10.0	HSCH ₂ (Cysteine)	19.4
CH ₃ (Alanine)	23.0	(Cystine)	19.6
(CH ₃) ₂ CH (Valine)	18.3	CH ₃ SCH ₂ CH ₂ (Methionine)	21.2
(CH ₃) ₂ CHCH ₂ (Leucine)	21.8	(Proline)	34.7
CH ₃ CH ₂ CH(CH ₃) (Isoleucine)	18.9	CH ₃ O ₂ CCH ₂ (Methylaspartate)	19.3
C ₆ H ₅ CH ₂ (Phenylalanine)	20.9	NH ₂ =C(NH ₂)NH(CH ₂) ₃ † (Arginine . HCl)	21.6
<i>p</i> -HO . C ₆ H ₄ CH ₂ (Tyrosine)	20.4	NH ₃ (CH ₂) ₄ ‡ (Lysine . HCl)	21.9
HOCH ₂ (Serine)	15.8	(Histidine . HCl)§	22.2

The chemical shifts are included in ref. 21.

Solutions were 3–4 M in H₂O, pH adjusted in the range 0.6–1.5.

* Chemical shifts in p.p.m. (± 0.3) downfield from $^{15}\text{NH}_4^+$ resonance of 5 M $^{15}\text{NH}_4^{15}\text{NO}_3$ in 2 N HNO₃ (see ref. 22 for this choice).⁴ The NO₃⁻ resonance is 354.1 p.p.m. to low field.

† Guanidine resonances at 43.3 (NH₂) and 54.8 (HN) p.p.m.

‡ Additional NH₃ resonance at 5.8 p.p.m.

§ Imidazole resonances at 144.2, 146.3 p.p.m.

with a higher operating magnetic field (4.2 T) may be used to bring lower sensitivities within range for either ^{13}C or ^{15}N provided the samples are not limited in amount. Sensitivity gains of between 15 and 20 which have been achieved for ^{15}N in this way are reported here. Sample concentrations may now be reduced and material of higher molecular weight may be studied. Additionally various new experiments have become accessible, which can give useful information about motional as well as structural characteristics. (A very elementary exposition of the basic NMR experiment is given in ref. 10.)

Lippmaa *et al.*¹¹ have found long (50–500-s) spin-lattice relaxation times (T_1) for ^{15}N in a range of small organic molecules. Where spectra are to be accumulated by repetitive pulse-Fourier transform methods, saturation of the resonances is then very easy, and can result in reduced signal intensity. This problem may be overcome by the addition of paramagnetic material to the sample⁶ to shorten T_1 , or by the use of long delays ($> T_1$) between pulses. We have now found that for larger molecules the problem of long T_1 s is not so serious (see below). (A useful introduction to relaxation times is ref. 12.)

The nuclear Overhauser effect (NOE) (reviewed in refs 12–14) is a well known intensity effect that occurs in double resonance experiments¹⁰. For ^{15}N , as for other nuclei, it can be defined as the ratio of the proton-decoupled ^{15}N signal intensity (I_D) to the proton-coupled intensity (I_0). If the motion of the molecules is fast enough (small correlation time, τ_c) then in the absence of exchange effects on T_1 , the NOE (denoted by $1 + \eta$) is given by

$$\frac{I_D}{I_0} = 1 + \eta = 1 + \frac{\gamma(^1\text{H})T_{1D}^{-1}}{2\gamma(^{15}\text{N})T_1^{-1}} \quad (1)$$

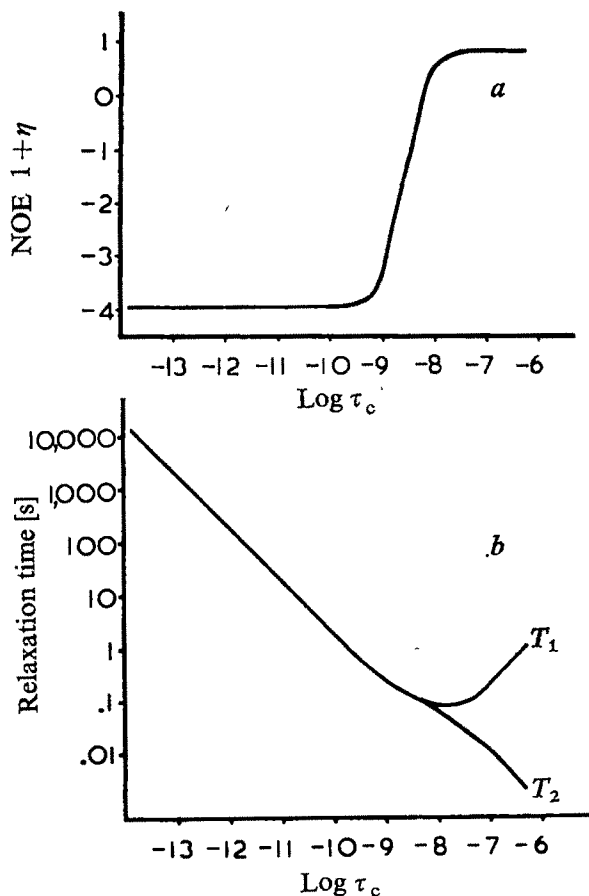
where T_{1D} is the ^{15}N - ^1H dipole-dipole contribution to the total ^{15}N relaxation time, T_1 . Since the signs of the magnetogyric ratios (γ) of ^{15}N and ^1H are opposite ($1 + \eta$) can be negative^{4,7}. The maximum possible NOE, which occurs for the 100% dipole-dipole case, is in fact -3.9. An inverted absorption mode signal then results. Other mechanisms for relaxation

for example, spin-rotation, chemical shift anisotropy^{11,13,14} may, however, compete with the dipole-dipole (that is, $T_{1D} > T_1$) thus reducing the NOE. In unfavourable situations $1 + \eta \rightarrow 0$, and zero ^{15}N signal results⁷. Again, addition of paramagnetic material to the sample may be used, this time to provide a dominant electron-nucleus (^{15}N) relaxation mechanism⁶. Then $T_1 \ll T_{1D}$ giving $1 + \eta \rightarrow 1$. An alternative way of suppressing the NOE which has been used frequently for ^{13}C is the gated decoupling technique¹⁵. We report here its first use for proton-decoupled ^{15}N systems to give ^{15}N resonances decoupled but without NOE.

The strength of the dipole-dipole mechanism depends on the inverse sixth power of the ^{15}N - ^1H internuclear separation and so it is most efficient for ^{15}N with directly bonded protons. To obtain inverted absorption mode ^{15}N signals (that is a significant NOE), however, it is not necessary that the protons be directly bonded, as has been shown by Lichter and Roberts⁷ on nicotine and Katz *et al.*⁸ on pheophytin a. What is necessary is merely that other relaxation mechanisms for ^{15}N should not be significant relative to the dipole-dipole.

If the approximation of fast motion does not hold, however, reduced NOEs can still result even if the dipole-dipole mechanism dominates. Allerhand and Oldfield¹⁶ have shown this for ^{13}C - $\{^1\text{H}\}$ experiments. (I - $\{S\}$ signifies observation of the I spins with saturation of the S spin transitions.) We report a similar analysis for the ^{15}N - $\{^1\text{H}\}$ system (Fig. 1a). Figure 1b shows a plot of T_1 and T_2 for a single ^{15}N - ^1H interaction, with a magnetic field of 2.1 T and internuclear distance, $r = 1.03$ Å. The expected reduction in the NOE occurs also for ^{15}N , and most importantly the sign of the signal changes as τ_c changes. In the region of fast motion (characterised by the inequality, $\omega^2\tau_c^2 \ll 1$, where ω is the resonance frequency) the fully inverted signal is obtained. When, however, $\omega^2\tau_c^2 \gg 1$, the signal is no longer inverted and the NOE reaches a limiting value of +0.88. At intermediate values of τ_c (about 6×10^{-9} s) signal

Fig. 1 a, Theoretical maximum ^{15}N - $\{^1\text{H}\}$ NOE. ^{15}N at 9.12 MHz; b, ^{15}N - ^1H dipole-dipole relaxation time. ^{15}N at 9.12 MHz.



nulling occurs. This motional nulling is to be contrasted with the original nulling condition we mentioned: the competitive relaxation situation.

We have observed a third nulling situation when off-resonance single frequency decoupling¹⁰ is used. Such experiments have proved to be very useful in overcoming assignment difficulties, notably in ¹³C NMR¹⁷. This possibility, and even a fourth nulling condition resulting from the gated decoupling experiment, have recently been discussed briefly¹⁸.

Table 2 ¹⁵N chemical shifts of some N-formyl and N-acetyl amino-acids

R	R HO ₂ C-CH-NHCHO	R HO ₂ C-CH-NHCOCH ₃
	N-Formyl δ ¹⁵ N 3-4 M in DMSO*	N-Acetyl δ ¹⁵ N 1 M in DMSO
H (Glycine)	93.7	89.4
CH ₃ (Alanine)	108.7	104.4
(CH ₃) ₂ CH (Valine)	101.7	98.0
CH ₃ CH ₂ CH(CH ₃) (Isoleucine)	102.5	99.0
(CH ₃) ₂ CHCH ₂ (Leucine)	106.0	101.7
C ₆ H ₅ CH ₂ (Phenylalanine)	104.4	100.9
HSCH ₂ (Cysteine)	—	99.0
CH ₃ SCH ₂ CH ₂ (Methionine)	104.7	—
Proline	115.3† 113.7†	110.8‡ 114.0§ 114.6¶ 96.4
β-Alanine	—	—

Chemical shifts in p.p.m. (±0.3) downfield from ¹⁵NH₄⁺ resonance of 5M ¹⁵NH₄⁺¹⁵NO₃.

* These data are included in ref. 21.

† *cis* and *trans* isomers.

‡ Both isomers (unresolved at 9.12 MHz).

§ *trans* isomer in MeOH-d₄.

¶ *cis* isomer in MeOH-d₄.

Nitrogen is an atom which often participates directly in chemical exchange phenomena. The ¹⁵N spectrum of a nucleus exchanging between two or more chemically shifted sites may display resonances due to each particular environment (slow exchange), a single averaged resonance (fast exchange), or at intermediate exchange rates much broadened resonances which are difficult to observe. Even if the sites are equivalent, exchange may affect the spectrum by causing decoupling of ¹⁵N from an attached labile proton. Assignment can then not be made by observation of multiplicities.

Contrary to first assessments¹⁹, it should not be thought that the NOE of a nitrogen attached to a labile proton need necessarily be affected by the exchange, simply because the multiplicity is lost. In other words, the single upright resonances in single resonance spectra may be inverted by double irradiation of the sample. We have observed this behaviour for the amino resonances of viomycin sulphate, for which the amido portions exhibited normal multiplicities (unpublished results of L. F. Farnell, G.E.H. and E.W.R.). The original expectation of an exchange effect on the NOE came from the possibility that modulation of the scalar ¹⁵N-H interaction (which gives rise to the multiplicity) could affect *T*₁ and thus the NOE. A dominant mechanism of this sort could give a value 1+η = +10.86. Leipert and Noggle have shown, however, that even when the scalar relaxation is at a maximum it is most unlikely to dominate²⁰.

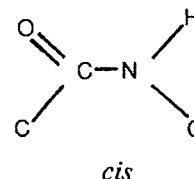
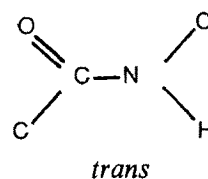
On the other hand, it is conceivable that exchange could affect the NOE by a new consideration. Suppose we have fast exchange of ¹⁵N between inequivalent sites, one can then obtain

the average NOE. Nulling could then occur if in the slow exchange limit the signals are opposite in sign.

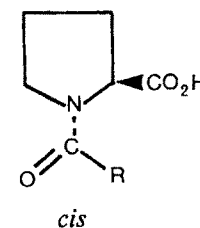
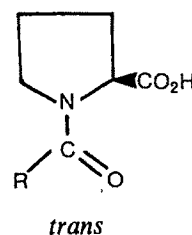
Applications to peptides

As a necessary preliminary to studying larger peptides we have investigated the types of structural information which may be obtained by ¹⁵N NMR work at natural abundance on small model systems; moreover systems wherein complicating effects of chemical exchange at nitrogen are minimal. Tables 1 and 2 show the ¹⁵N chemical shift data for some amino acid methyl ester hydrochlorides (some of which have been the subject of a preliminary communication⁹) and some N-acyl amino acids. The chemical shift variations which we have so far observed are due almost entirely to structural changes because medium effects have been shown to be small. For example a pH study on glycine methyl ester hydrochloride showed the ¹⁵N shift was insensitive to pH change in the range 0-4. Similarly the N-formyl derivatives (Table 2) showed a negligible shift with concentration in the range 1-4 M in DMSO, and for the N-acetyl derivatives the effect of changing the solvent from DMSO to methanol is to impart only a small (1.3±0.5 p.p.m.) downfield shift. The cause of this last small effect is probably the differing intermolecular hydrogen bonding situations in the two solvents.

For each example shown in Table 2, a single ¹⁵N resonance was generally observed and is due²³ to the *trans* amide form.



Resolution of the *cis* and *trans* resonances of N-acetyl L-proline in DMSO (about 71% *trans* in DMSO solution²⁴) was achieved only at the higher resonance frequency (18.24 MHz, 4.2 T) and not at 9.12 MHz (2.1 T). The shift difference was 0.3 p.p.m. with the *cis* signal being to higher field. This illustrates the second advantage, further to the sensitivity gain, of operating at higher frequency. For a solution in methanol-d₄ the solvent induced shift was sufficient to give resolved signals at 9.12 MHz: the signal for the *cis* isomer was 0.6 p.p.m. to low field.



The differential effects, amounting to about 3.5 p.p.m. (±0.3 p.p.m.), on ¹⁵N chemical shifts in amino acids by formylation and acetylation (Table 2) lead in theory to the possibility of obtaining sequence information for small peptides by ¹⁵N NMR. Table 3 shows the ¹⁵N chemical shifts of some dipeptide and a tripeptide N-acetyl derivatives, which were obtained to test this possibility. Consider first the series: AcGly, AcGly¹Gly², AcGlyAla, AcGlyLeu. The signal at about 89 p.p.m. for all must be due to Gly (Gly¹ for AcGly¹Gly²), and demonstrates that the introduction of the C-terminal amino acid unit has a negligible effect on the ¹⁵N shift. This is verified by the comparisons AcAla, AcAlaGly (Ala at about 104.5

Table 3 ^{15}N chemical shifts of some N-acetyl peptides

	Concentration* (M)	$\delta^{15}\text{N}^\dagger$	
Ac-Gly-Gly	1	89.1	84.0
Ac-Gly-Gly-Gly	0.8	89.7(1)‡	84.0(2)‡
Ac-Gly-L-Leu	1	89.1	96.4
Ac-L-Leu-Gly	1	102.2	84.6
Ac-Gly-L-Ala	1	89.1	98.8
Ac-L-Ala-Gly	1	104.7	83.5

*Solutions in $\text{DMSO}-d_6$.† Chemical shifts in p.p.m. (± 0.3) downfield from NH_4^+ resonance of $5\text{M } ^{15}\text{NH}_4^{15}\text{NO}_3$.

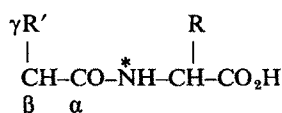
‡ Numbers in parentheses are relative signal intensities.

p.p.m.) and AcLeu, AcLeuGly (Leu at about 102 p.p.m.).

The introduction of an N-terminal amino acid unit does, however, shift the ^{15}N resonance of the C-terminal unit (in the dipeptides) away from the acetyl amino acid position as shown by the comparisons:

AcGly	AcGlyGly	AcAlaGly	AcLeuGly
89.4	84.0	83.5	84.6 p.p.m.

The shift seems to be insensitive, however, to the structure of the N-terminal amino acid unit. This is reasonable, since a change in the N-terminal unit is a γ or δ effect across a carbonyl group:



Thus the ^{15}N chemical shift of glycine, for example, in a peptide chain would be expected to be about 84 p.p.m. ± 0.5 p.p.m. at least in DMSO solution. This is verified by the shifts for N-acetyltriglycine:

Ac-Gly-Gly-Gly
89.7 84.0 84.0 p.p.m.

In the same way that glycine in a peptide gives a ^{15}N shift about 5.4 ± 0.5 p.p.m. upfield from N-acetyl glycine, so also the alanine in AcGlyAla gives a shift 5.6 p.p.m. upfield from the value for AcAla, and leucine in AcGlyLeu is 5.3 p.p.m. upfield from AcLeu. Thus, the theoretical promise of getting sequence information is in practice not realised for these derivatives in DMSO solution. The relative numbers and the nature of the peptide components are, however, easily obtained.

^{15}N study of gramicidin S

The naturally occurring, antibiotic, cyclic decapeptide gramicidin S, molecular weight 1,120, has the primary structure cyclo-(-D-Phe-L-Pro-L-Val-L-Orn-L-Leu) $_2$ and has been studied extensively as a model for proteins by both proton²⁵ and ^{13}C NMR²⁶⁻²⁸. Its relatively high solubility in both methanol and DMSO makes it a suitable substrate for testing the applicability of ^{15}N NMR at the natural abundance level to higher molecular weight material. Spectra obtained at 2.1 T are shown in Fig. 2

Table 4 ^{15}N chemical shifts for gramicidin S

Solvent	Concentration	$\delta^{15}\text{N}^*$		
Methanol	0.2 M	11.5	97.4	105.2
		115.6		
DMSO-methanol (4:1)†	0.25 M	14.2	93.4	103.3
		106.5	114.0	
DMSO	0.3 M	16.1		

* Chemical shifts in p.p.m. (± 0.3) downfield from NH_4^+ resonance of $5\text{M } ^{15}\text{NH}_4^{15}\text{NO}_3$.

† Sample prepared by dilution of 0.3 M solution in DMSO by methanol.

(the chemical shifts are summarised in Table 4) and illustrate the excellent resolution obtainable in spite of the relatively long accumulation times required. The backbone amide nitrogens resonate in the region 93–116 p.p.m. whereas the side-chain $-\text{NH}_2$ of ornithine is substantially to higher field. That a spectrum in DMSO solution (Fig. 2c) showed no amide nitrogen resonances is noteworthy. It is the first example of nulling of ^{15}N resonances by the restricted motional effects outlined above.

To confirm this proposal, ^{13}C NMR spectra of gramicidin S at 22.6 MHz were recorded, in various solvent and concentration conditions with ^1H decoupling, and the half-widths of various well resolved resonances were measured (Table 5). The full resonance width at half height ($\Delta\nu_i$ Hz) is related to the spin-spin relaxation rate, T_2 , by $1/T_2 = \pi\Delta\nu_i$ (s^{-1}). From these data, effective correlation times (τ_c) for overall reorientation of gramicidin S were calculated (Table 5). The correlation times obtained for solutions 0.2 M in methanol and 0.3 M in

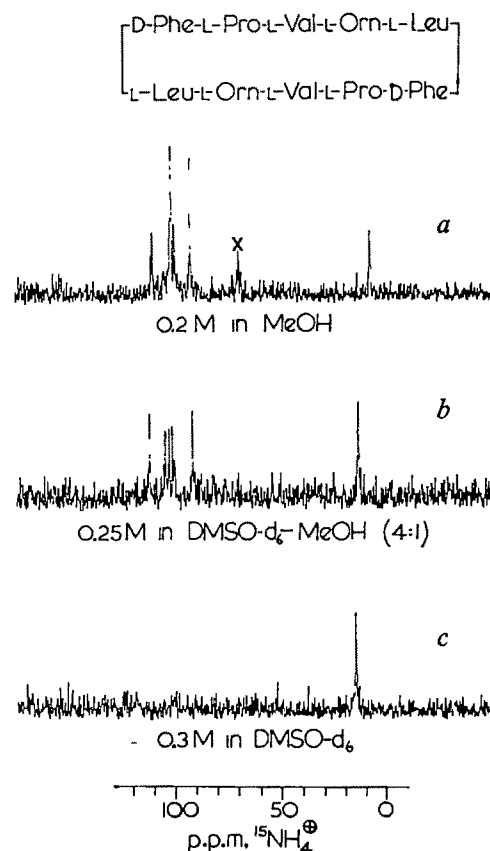


Fig. 2 ^1H Noise decoupled natural abundance ^{15}N NMR spectra (9.12 MHz) of gramicidin S. Average accumulation time per spectrum was about 36 h. These are magnitude spectra and thus contain no information on the sign or magnitude of the NOE. X marks the position of coherent noise from the deuterium lock.

DMSO were $(6 \pm 3) \times 10^{-10}$ s and $(5.4 \pm 0.8) \times 10^{-9}$ s, respectively. (The first value agrees with that obtained by Allerhand and Komoroski²⁷ from ^{13}C T_1 measurements on a more dilute solution of gramicidin S in methanol: $\tau_c = (3.3 \pm 0.2) \times 10^{-10}$ s.) Use of these data in Fig. 1 shows that the ^{15}N resonances from the 0.2 M solution in methanol must have a near maximum NOE ($1 + \eta = -3.9$ to -3.6), whereas those from the 0.3 M solution in DMSO are in the nulled region.

Further confirmation that the ^{15}N resonances from the solution of gramicidin in DMSO were nulled by the NOE was provided by two experiments at 18.2 MHz: a variable temperature experiment and a gated decoupling experiment. The ^1H decoupled spectrum of gramicidin (0.15 M in DMSO) at about 18 °C showed no amide resonances and a strong NH_2 reso-

Table 5 Average ^1H decoupled ^{13}C resonance half widths for gramicidin S

Carbons	0.2 M in methanol- d_4	0.23 M in DMSO- d_6 /methanol- d_4	0.23 M in DMSO- d_6	0.3 M in DMSO- d_6
Methyls	NR	3	4	6
Methine C	5	6	9	14
Carbonyl	2	2	2	4
$\tau_c \times 10^{10}$ s	6 ± 3	10 ± 5	22 ± 7	54 ± 8

Full line widths at half height (± 1.2 Hz) are estimated to include about 1 Hz instrumental broadening. NR, not fully resolved—overlapping resonances.

nance (Fig. 3b). An increase of sample temperature from 18 °C to 47 °C decreased the correlation time sufficiently to quench the nulling and give amido resonances showing a partial NOE, as can be seen in Fig. 3. In the gated decoupling experiment the conditions were chosen to yield decoupled resonances without NOE and the spectrum in Fig. 3b was thereby made to yield amido resonances opposite in phase to those obtained at 47 °C. These experiments were feasible only because of the increased sensitivity at 18.24 MHz.

In every ^{15}N spectrum (at 9.12 and 18.24 MHz) the side chain NH_2 resonance of ornithine was observed. Here, the significantly greater mobility of the side chain²⁷ relative to the backbone of the molecule ensures a sufficiently short correlation time to preclude signal nulling.

From the shift data presented above (Tables 2 and 3) it is possible to make a full assignment for the amide nitrogen resonances of gramicidin S. To a first approximation the data in Table 4 predict the amide nitrogen resonance of a given amino acid in a peptide chain to be independent of its position in the chain but to be influenced strongly by its substituents at the α carbon. We have not observed the amide nitrogen of ornithine in a peptide chain elsewhere but its shift can be calculated approximately⁵. Thus the relative amide nitrogen chemical

Fig. 3 ^1H Noise decoupled natural abundance ^{15}N NMR spectra (18.24 MHz) of gramicidin S (0.18 M in DMSO). Average accumulation time per spectrum was about 40 min. Both spectra are out of phase by 180°, that is, signals are inverted by the NOE. a, 47 °C; b, 18 °C.

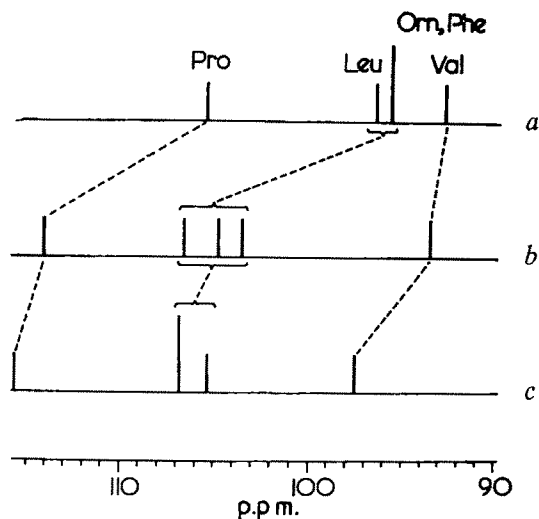
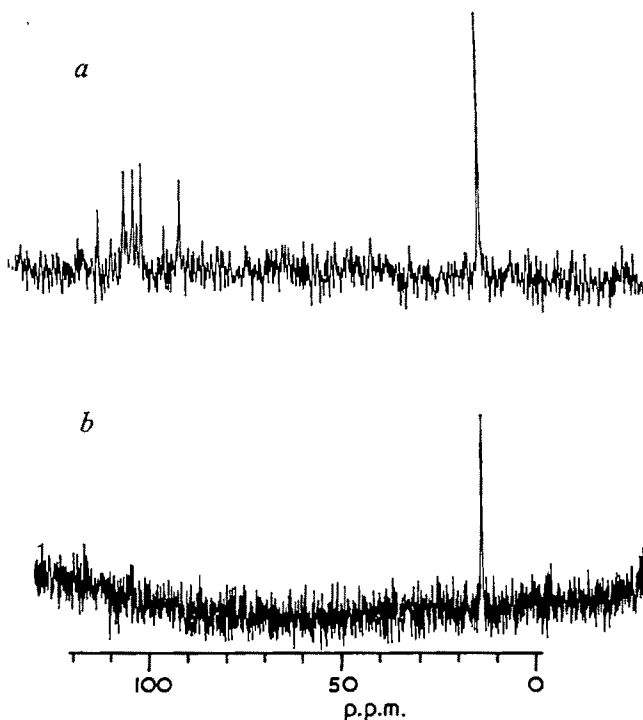
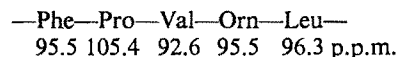


Fig. 4 Correlation diagram of amide nitrogen resonances from gramicidin S. a, Predicted from model dipeptides (see text); b, observed from solution 0.23 M in DMSO; c, observed from solution 0.2 M in methanol

shifts of gramicidin S are predicted as the acetyl amino acid shifts (Table 2) minus 5.4 p.p.m. (Table 3). (This actually gives the amide nitrogen resonance as the C-terminal unit of an N-acetyl dipeptide.)



Although the patterns of observed and predicted chemical shifts are similar, the predicted ones are each substantially to higher field than the observed shifts as shown by the correlation diagram (Fig. 4). The reason for this is not yet clear, but it should be remembered that the predictions are from model dipeptides which include free carboxylic acid groups, whereas gramicidin S has side chain $-\text{NH}_2$ groups. A second possible source for the displacement in Fig. 4 is the unknown effect of constraining the peptide in a cyclic structure and introducing intramolecular hydrogen bonding between leucine and valine²⁵. Each of these effects is the subject of a continuing study.

For our study the amino acid and small peptide derivatives were prepared by standard methods. We used gramicidin J from *Bacillus brevis* (nagano) (Sigma) which has the same chemical structure as gramicidin S. ^{15}N NMR spectra were obtained from the Bruker HFX-90 (^{15}N at 9.12 MHz) and Bruker WH-180 (^{15}N at 18.24 MHz) instruments operating in the pulse-Fourier transform mode with ^1H noise decoupling. Samples were contained in tubes of outer diameter 10, 13 or 15 mm (HFX-90) and 25 mm (WH-180). Deuterium in the solvent or, in the case of aqueous (H_2O) or methanol (CH_3OH) solutions, deuterium oxide in a concentric 5-mm tube, provided the field-frequency stabilisation signal. D_2O and CD_3OD were not normally used as solvent in order to prevent exchange of deuterium for labile protons in the solute. Typical spectral accumulation times (HFX-90) were 5–10 h for 1-M solutions of amino acid derivatives with pulse flip angles about 30°, and pulses were repeated every 0.4 s. The higher magnetic field, larger sample volume and quadrature detection system of the WH-180 instrument provided an estimated increase in signal-to-noise of about 18:1 for a single pulse experiment, or a saving in time corresponding to a factor of about 300 for spectral accumulation, when compared with the HFX-90 even with 15-mm tubes.

The accuracy of the measured ^{15}N chemical shifts was limited by the digitisation of the transformed spectrum (2.4 Hz per point) and was about 0.3 p.p.m. for the HFX-90 and 0.15 p.p.m. for the WH-180. ^{15}N Chemical shifts were referenced to the

ammonium ion resonance from an external sample of $^{15}\text{NH}_4^+$ $^{15}\text{NO}_3^-$ 5 M in 2-N nitric acid.

^{13}C Spectra at 22.6 MHz were obtained with the HFX-90 instrument. Sample temperatures for the HFX-90 instrument were about 35 °C, and for the WH-180 instrument are estimated to be accurate to ± 3 °C.

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letters to nature

Clustering of faint blue objects

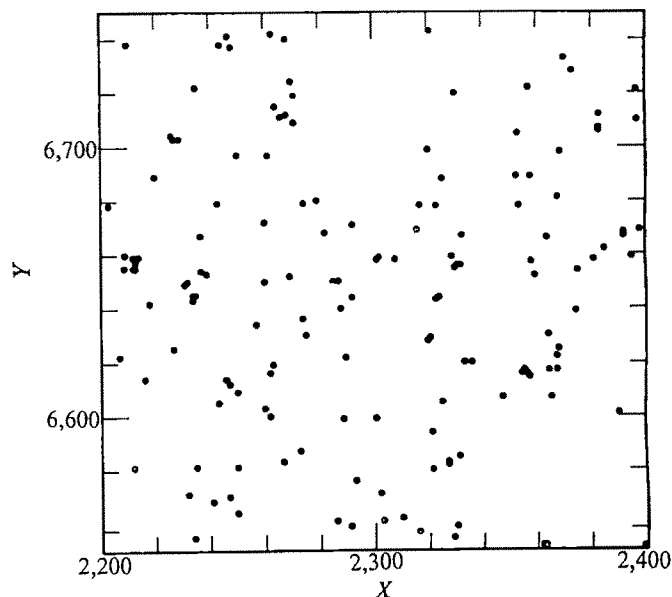
PHOTOGRAPHS taken with the UK 48-inch Schmidt Telescope at Siding Spring, Australia using hypersensitised Kodak IIIaJ emulsions, record objects as faint as magnitude 23 in exposures of an hour. Most galaxies on the photographs are very faint and at, say 22 mag probably have redshifts $Z \sim 0.5$. At such large redshifts the K term is responsible for considerable reddening ($B-V \simeq +2$ mag) and it may perhaps not be expected that such blue sensitive emulsions would show much increase in the number of faint galaxies recorded as the limiting magnitude increases beyond about 21 mag. The number of objects does, however, seem to continue to increase significantly with limiting blue magnitude and the question therefore arises as to whether or not a substantial number of faint images are blue and if so, what objects do they represent and how are they distributed?

To examine this question a IIIaJ photograph of a region near the south Galactic pole has been compared with a red-sensitive photograph of the same region. Both plates reach very faint magnitudes and when viewed in a Zeiss comparator most images are similar on both plates—that is to say, are of neutral apparent colour. The outer parts of some relatively bright spirals are noticeably blue, the inner parts neutral. Among the faint objects (which are the vast majority) a significant minority, several per cent, are noticeably blue, and a smaller minority are noticeably red.

The positions of noticeably blue objects in an area 22 arc min square were measured by one of us (V.C.R.) and the distribution is shown on Fig. 1. There are 168 objects. Only objects for which images appeared on both red and blue plates were recorded in order to avoid the accidental inclusion of plate faults. The criterion of 'blueness' by which they were chosen in this way is of course arbitrary and subjective. They represent about 10% of all the images in that area of the photographs: that is to say they are the bluest 10% of the objects. At the magnification employed, $\times 13$, almost all the images appear stellar. Comparison with a similar IIIaJ photograph of the cluster Kron 3 near the SMC in which M. F. Walker has determined electronographic magnitudes to $B = 22.7$ shows that many, perhaps most, of the objects are $B = 22$ or fainter and the great majority are fainter than $B = 21$.

It is immediately apparent from Fig. 1 that the apparent surface distribution is non-random. There is a tendency to clustering and particularly for the occurrence of pairs. This is confirmed by Fig. 2, which shows the frequency distribution of the separations of each object from its nearest neighbour (histogram $O(V.C.R.)$). A Poisson distribution with the same average separation (45'') is shown, $P(45,168)$, for comparison. If the objects were uniformly distributed the average separation would be 100'' and so the observed distribution represents a concentration by a factor of five. It has a sharp peak at a much smaller separation than the average, at 10–20'', and a second broad maximum around 70''. It is fairly well represented by the sum of two Poisson distributions $P(20,102;77,66)$ in which two thirds of the objects have a mean separation of 20'' and the remaining third a mean separation of 77''. The first of these

Fig. 1 The apparent distribution of faint blue objects as measured by V.C.R.



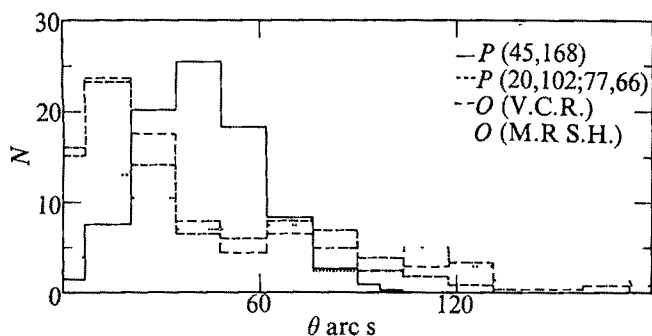


Fig. 2 N is the number of faint blue objects per unit of $7''$ in θ . θ is the angular separation of each from its nearest neighbour. O (V.C.R.) is the distribution measured by V.C.R. corresponding to Fig. 1; O (M.R.S.H.) that measured by M.R.S.H. corresponding to Fig. 3. The latter's threshold was chosen more blue than the former's and thus the total number of objects is smaller (73 as against 168). $P(45,168)$ is a Poisson distribution with the same mean θ (45) and total N (168) as O (V.C.R.). $P(20,102;77,66)$ is the sum of two Poisson distributions with mean θ 20 and 77'' and total numbers 102 and 66 respectively.

distributions represents the preponderant number of pairs evident on Fig. 1.

In order to check the validity of the data, especially in view of its element of subjectivity, and to provide data for another region, M.R.S.H. similarly measured the positions of the bluest objects in a second region of the same size but separated by several degrees from the first. His result is shown on Fig. 3. The number of objects is smaller, 73 against 168, and it appears from a cross check by the two of us that M.R.S.H. set his

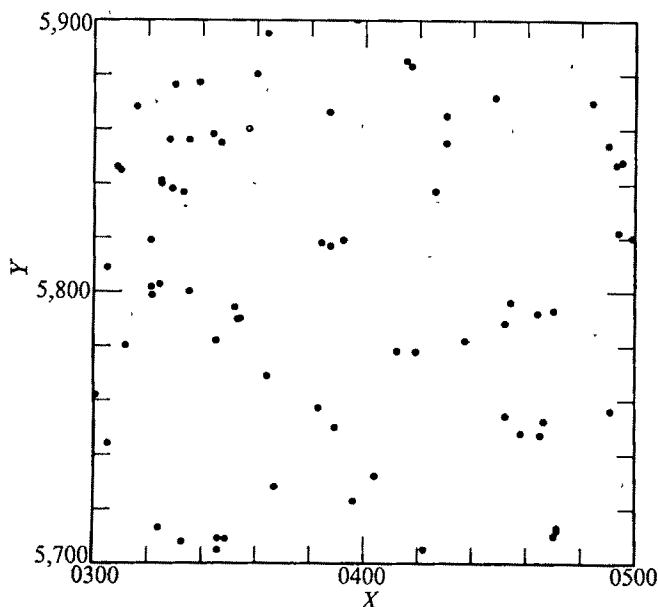


Fig. 3 The apparent distribution of faint blue objects as measured by M.R.S.H. in a region several arc degrees away from Fig. 1.

criterion at a bluer level than V.C.R.; thus Fig. 3 represents the distribution of the bluest 5% of the images. The same effects are again immediately apparent, however—some clustering and a preponderance of pairs. This impression is also confirmed by the histogram O (M.R.S.H.) in Fig. 2.

The non-random nature of the distribution makes it very unlikely that the objects are faint blue stars in the Galactic halo; they are almost certainly extragalactic. The faintness and star-like quality of the images indicate that they are distant, perhaps very distant, and compact. The blueness suggests that they are

not ordinary galaxies but lie in the range from very blue compact galaxies to quasars. Whatever they are the high degree of clustering, and especially the preponderance of pairs, appears to be significant. Taken together with a recent indication of an increase in the degree of clustering of galaxies with distance¹ this may be further direct evidence of an evolving universe.

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Possible local variation of the Hubble constant in van den Bergh's calibration of Sc-type galaxies

IN an important series of papers for the determination of the Hubble constant, Sandage and Tammann¹⁻⁶ have concluded that the Universe is expanding in a remarkably uniform and isotropic way, with Hubble constant (H) $55 \pm 5 \text{ km s}^{-1} \text{ Mpc}^{-1}$. If true, this result is evidently crucial for all future cosmological models. As a consequence it should be tested and analysed in all possible ways. Their low H value is evidently based on their recalibration of the absolute magnitudes of the various luminosity classes of Sc-type galaxies based on two samples of nearby, and one sample of distant galaxies (Tables 3 and 4, ref. 5, and the Table, ref. 6).

Here we discuss some consequences of Sandage and Tammann's specific choice of absolute magnitudes, and to compare them with the calibration of van den Bergh⁷, which had been generally accepted in the literature.

Two preliminary remarks should be made here. First, starting from the data and calibration of Sandage and Tammann, de Vaucouleurs has analysed possible variations of the redshift distribution with supergalactic longitude and latitude, and discovered significant deviations from isotropy. Second, if one calculates H using only the Sc I galaxies, one does indeed

Table 1 Recalibration of Sandage and Tammann's data on sources in local supergalaxy anticentre direction using van den Bergh's absolute magnitudes

Sc classes	M_{ST}	M_{vdb}	$\alpha = H_{vdb}/H_{ST}$
I	-21.25	-20.0	1.778
I-II	-20.74	-19.7	1.614
II	-20.23	-19.4	1.466
II-III	-19.72	-18.9	1.459
III	-19.21	-18.3	1.521

find the same H at all distances, although Sandage and Tammann's unbiased data for Sc II anticentre galaxies (Tables 3 and 4, ref. 5), yield $\langle H \rangle = 77.6 \pm 6.0 \text{ km s}^{-1} \text{ Mpc}^{-1}$, which differs significantly from the result for Sc I anticentre galaxies (Table 2) $\langle H \rangle = 61.4 \pm 6.8 \text{ km s}^{-1} \text{ Mpc}^{-1}$ so that $\Delta H = 19.2 \pm 8.1 \text{ km s}^{-1} \text{ Mpc}^{-1}$ (Student's $t = 2.53$).

If, then, one accepts de Vaucouleurs' result (unpublished), that the overall motion of the local supergalaxy contributes significantly to the redshift distribution, one should concentrate discussion of the possible consequences for H determination of a recalibration of Sandage and Tammann's samples with van den Bergh's absolute magnitudes, on sources located in the anticentre of the local supergalaxy direction.

We do this in Table 1. Column 1 shows the Sc classes used in Sandage and Tammann's determination of H ; columns 2

Table 2 Comparison of values of H obtained for the new calibration, for the two samples described in the text

Sc-type	H_{ST} km s ⁻¹ Mpc ⁻¹	H_{vdb} km s ⁻¹ Mpc ⁻¹	H_{ST} km s ⁻¹ Mpc ⁻¹	H_{vdb} km s ⁻¹ Mpc ⁻¹
Sc I	61.40 ± 6.84	109.16 ± 12.16	51.73 ± 2.72	91.98 ± 4.83
Sc I-II	67.83 ± 9.59	109.48 ± 15.5		
Sc II	77.61 ± 5.99	113.70 ± 8.82		

and 3, the values of the corresponding absolute magnitudes used by them and by van den Bergh, column 4, the numerical factors by which the $H = v/r$ values of Sandage and Tammann should be multiplied, to pass to the corresponding values in van den Bergh's calibration, that is α in $H_{vdb} = \alpha H_{ST}$.

The calculations have been performed on all the unbiased anticentre galaxies in refs 5 and 6. For nearby galaxies we have used exactly the limit proposed by Sandage and Tammann to eliminate a possible bias, that is, for those with values of $\log v \leq 3.3$. The same has been done for distant Sc I galaxies—we have restricted the calculation (as prescribed by Sandage and Tammann) to the 40 anticentre objects with $\log v \leq 3.92$.

The results are summarised in Table 2. Column 1 shows the Sc classes analysed; columns 2 and 3, the corresponding H values obtained in calibrations by Sandage and Tammann and van den Bergh for the sample of 7 Sc I, 4 Sc I-II and 8 Sc II nearby unbiased anticentre galaxies. Columns 4 and 5 contain the respective H values for the unbiased sample of 40 distant anticentre Sc I galaxies.

We derive three conclusions from Table 2. The first is that Sandage and Tammann's calibration yields significant differences in the $\langle H \rangle$ values obtained with different types of nearby Sc galaxies—a fact difficult to reconcile with their own assumptions. The second is that van den Bergh's calibration, though possibly open to criticism, does not present this inconvenience, and yields a consistent $\langle H \rangle$ value for all classes of nearby Sc galaxies: $\langle H \rangle = 111.2 \pm 6.3$ km s⁻¹ Mpc⁻¹, for 19 objects. The third is that this latter value differs significantly from the distant Sc $\langle H \rangle$ value (that is, $\langle H \rangle = 92 \pm 5$ km s⁻¹ Mpc⁻¹) since we have $\Delta H = 19.2 \pm 8.1$ km s⁻¹ Mpc⁻¹.

If one accepts van den Bergh's calibration, this evidently yields a statistically significant decrease of H with distance, since for the hypothesis $H(\text{nearby}) = H(\text{distant})$, $t = 2.38$ ($P < 0.05$). Finally it should be noted that the existence of such a variation of H is independently supported by very recent determinations of the distance D of 29 Sc-type galaxies (12 of which belong to the preceding Sandage-Tammann sample) made on the 21-cm line at the Nançay Radiotelescope by Durand⁸ and Bottinelli *et al.* (unpublished). Indeed, if one divides Durand's result into two groups: the first with 15 objects at $D \leq 30$ Mpc and the second with 14 objects at $D > 30$ Mpc, one obtains $\langle H \rangle_1 = 95.2 \pm 11.0$ and $\langle H \rangle_2 = 63.5 \pm 5.4$ km s⁻¹ Mpc⁻¹.

Obviously, this possible variation of H with distance can be correlated with the angular anisotropy of H observed by Rubin, Ford and Rubin⁹, which has been confirmed on various types of source¹⁰. Independently of any specific interpretation (such as a variation of the redshift of photons passing through the radiation field of distant galactic clusters¹⁰, this conflict between the results of calibrations on the same sample by Sandage and Tammann and van den Bergh shows that further observational work needs to be done on this calibration problem.

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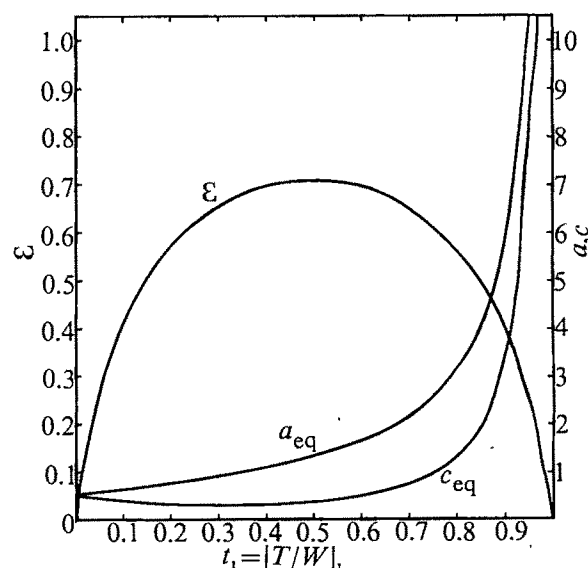
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Why there are no elliptical galaxies flatter than E7

It has long been known¹⁻³ that the flattest elliptical galaxies observed are of type E7, corresponding to an axial ratio of 3.33:1 whereas SO and spiral galaxies are seen with axial ratios up to 20:1. We show, using Maclaurin spheroid models, that an initially spherical protogalaxy, composed entirely of stars, will always relax after a dissipationless collapse to an equilibrium configuration with $\epsilon \equiv 1 - (c/a) \leq 0.70925$, where a, c are the semi-major, semi-minor axes. This happens regardless of original angular momentum of the galaxy, and is in remarkable agreement with the maximum observed flattening of elliptical galaxies.

According to the standard cosmological picture, protogalaxies begin as small density perturbations at recombination, expand to a maximum radius and recollapse, reaching the maximum collapse point at $t = T_c$. By $t = T_c/2$, the protogalaxy has acquired its angular momentum by tidal interaction⁴ or turbulence⁵. At this time, we assume the protogalaxy to be a uniformly dense sphere, of mass M , of initial radius a_i

Fig. 1 Ellipticity ϵ , semi-major and semi-minor axes (a, c) of the equilibrium elliptical galaxy as a function of $t_1 = |T_{rot}/W|$. The curve $\epsilon(t_1)$ is symmetrical with respect to $t_1 = 0.5$.



rotating uniformly with angular velocity Ω_i . There are initially no random velocities. To characterise the angular momentum of the protogalaxy, we introduce the convenient parameter t_i as the ratio of the rotational kinetic energy T_{rot} of the sphere to its potential energy W :

$$t_i = \left| \frac{T_{\text{rot}}}{W} \right| = \frac{\Omega_i^2 a_i^3}{3GM} \quad (1)$$

The initial binding energy of the protogalaxy is:

$$E_i = W_i (1 - t_i) = -\frac{3}{5} \frac{GM^2}{a_i} (1 - t_i) \quad (2)$$

If star formation occurs early and is completed by $t = T_c/2$, the protogalaxy undergoes a dissipationless collapse¹³, relaxes violently⁶ and settles into an equilibrium configuration by $t = 3T_c/2$ (ref. 7). Violent relaxation tends to isotropise random velocities, as suggested by an N -body calculation including infall and tidal effects⁸, and by fits to the light distributions of elliptical galaxies using King's⁹ models which have an isotropic distribution of random stellar velocities. We adopt as the simplest model for the equilibrium galaxy a Maclaurin spheroid in virial equilibrium with uniform rotation and isotropic pressure. Although real elliptical galaxies have dense cores and exhibit differential rotation the ellipticities predicted by numerical models of elliptical galaxies and Maclaurin spheroid models are in reasonable agreement¹³. The energy of the Maclaurin spheroid is

$$E_{\text{eq}} = \frac{W_{\text{eq}}}{2} = -\frac{3}{10} \frac{GM^2}{a_{\text{eq}}} \frac{\sin^{-1}e}{e} \quad (3)$$

where eq denotes 'equilibrium' and

$$e = \left[1 - \left(\frac{c}{a} \right)^2 \right]^{1/2}$$

Since there is no dissipation, $E_i = E_{\text{eq}}$ or

$$2(1 - t_i) = \frac{\sin^{-1}e}{e} \frac{a_i}{a_{\text{eq}}} \quad (4)$$

Angular momentum conservation gives

$$(T_{\text{rot}})_{\text{eq}} = \left[\frac{a_i}{a_{\text{eq}}} \right]^2 (T_{\text{rot}})_i = \frac{3}{5} \frac{GM^2}{a_i} t_i \left[\frac{a_i}{a_{\text{eq}}} \right]^2 \quad (5)$$

The rotational kinetic energy of a pressure supported equilibrium Maclaurin spheroid is¹⁰

$$[T_{\text{rot}}]_{\text{eq}} = \frac{3GM^2}{10a_{\text{eq}}e^3} [(3 - 2e^2) \sin^{-1}e - 3e(1 - e^2)]^{1/2} \quad (6)$$

Combining equations (4) to (6):

$$t_i(1 - t_i) = \frac{1}{4} \frac{\sin^{-1}e}{e^4} [(3 - 2e^2) \sin^{-1}e - 3e(1 - e^2)]^{1/2} \quad (7)$$

which for a given t_i , can be solved for e ; a_{eq} and c_{eq} are then found using equation (3) and (4). The results are shown in Fig. 1. Note that equation (7) is symmetrical about $t_i = 0.5$ which corresponds to an ellipticity of 0.70925. For $t_i < 0.5$ (probably in the majority of cases), the galaxy gets flatter on adding angular momentum, but past $t_i = 0.5$ adding angular momentum makes the equilibrium galaxy rounder. Given our model, no elliptical galaxy can be flatter than an E7, no matter how fast it rotates initially. This surprising fact is due to the equilibrium Maclaurin spheroid blossoming to infinite size with only a finite amount of angular momentum. At $t_i = 1$,

the galaxy has only $\sqrt{2}$ times the angular momentum of one at $t_i = 0.5$ (initially in virial equilibrium), but because the total kinetic energy has increased to equal the potential energy, the binding energy becomes 0, and a_{eq} and c_{eq} become infinite. For $t_i > 1$, the galaxy becomes unbound. Another symmetry about $t_i = 0.5$ is found in numerical experiments by Bouvier and Janin¹¹ who found that spherical systems with t_i (random) = 0.25 and t_i (random) = 0.75 have comparable amounts of violent relaxation. The system with the least violent relaxation is the E7 galaxy with $t_i = 0.5$.

Any initial random velocities, or any net expansion or contraction of the initial spherical protogalaxy, can only decrease the ellipticities, since all these effects decrease the binding energy without affecting the angular momentum; thus the upper limit of $\epsilon = 0.70925$ is left intact. Maclaurin spheroids become dynamically unstable at $|T_{\text{rot}}/W| = 0.273$ ($e = 0.95289$ or $e = 0.69668$ (ref. 11)). This is close enough to our maximum value of 0.70925 not to affect our conclusions. Real stellar systems with $|T_{\text{rot}}/W| \gtrsim 0.14$ ($e > 0.81267$ or $e > 0.41727$) may, however, be unstable to bar instabilities¹². Phase mixing may smear the bar out in time so as to leave a more centrally condensed axisymmetric galaxy with $|T_{\text{rot}}/W| \sim 0.14$. It is not clear, however, that the final ellipticity is changed greatly by this process.

Our result depends on the assumption of an initially spherical state and of conservation of energy and angular momentum. The final Maclaurin spheroid can be as flat as one wants if energy is dissipated—which we believe is the mechanism for the formation of a disk spiral galaxy¹³—or if the initial state is non-spherical. To investigate this, we write:

$$E_{\text{eq}} = \alpha E_i \quad (8)$$

$$[T_{\text{rot}}]_{\text{eq}} = \beta \left(\frac{a_i}{a_{\text{eq}}} \right)^2 [T_{\text{rot}}]_i \quad (9)$$

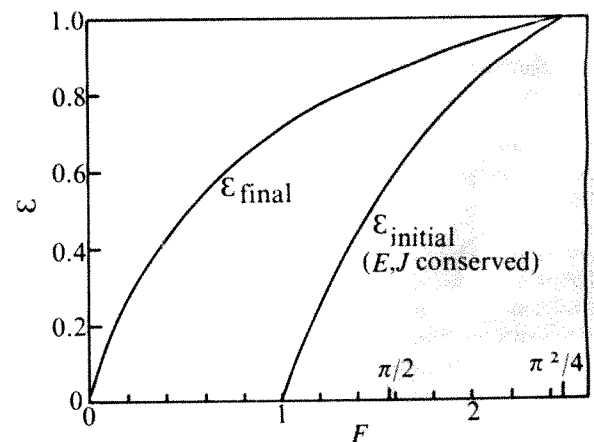
$$\text{and} \quad W_i = -\frac{3}{5} \frac{GM^2}{a_i} \frac{\sin^{-1}e_i}{e_i} = -\frac{3}{5} \frac{GM^2}{a_i} \gamma \quad (10)$$

This has the consequence of multiplying the left hand side of equation (7) by $\alpha\beta\gamma$. Let $F = 4\alpha\beta\gamma t_i(1 - t_i)$. Figure 2 shows ϵ_{final} as a function of F . $\epsilon_{\text{initial}}$ as a function of F is also shown in the case when the energy E and the angular momentum J are conserved. There are several points to be made:

(1) For the final state to be a flat disk $F = \pi^2/4$, so if J is conserved and the initial state is spherical then the dissipation must be large enough so that $E_f/E_i \geq \pi^2/4$.

(2) In the tidal interaction picture, the angular momentum

Fig. 2 Ellipticity ϵ_{final} of the equilibrium elliptical galaxy as a function of $F = 4\alpha\beta\gamma t_i(1 - t_i)$. The initial ellipticity ϵ_i is also given as a function of F when energy and angular momentum are conserved.



is gained over a time scale $\sim T_c/2$, so for $t_i \gtrsim 0.5$ the original spherical shape may become somewhat flattened in the process of gaining angular momentum, which would have the consequence of increasing the maximum ellipticity ϵ_m . For example, for $\epsilon_i = 0.5$, Fig. 2 shows that for a dissipationless collapse, $\epsilon_{max} \sim 0.83$ ($F = 1.48$). The limiting case is again given by $F = \pi^2/4$ when $\epsilon_i = \epsilon_m = 1$.

In conclusion, with a roughly spherical shape initially and a dissipationless collapse conserving angular momentum, we expect no equilibrium elliptical galaxies flatter than E7 regardless of the original angular momentum. The remarkable agreement of this with the maximum observed ellipticity would seem to argue that this very simple picture may be a close approximation to reality.

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Galactic dust lanes and lunar soil

It has been proposed by McCrea¹, Shapley² and Hoyle and Lyttleton³ that passages of the Solar System through interstellar clouds have appreciable effects on the Earth. McCrea argues that the recurrence of ice epochs⁴ every ~ 250 Myr coincides with the passage of the Solar System through a galactic spiral arm approximately every 10^8 yr. We report here studies on the character and grain-size distribution of texturally-mature lunar soils which support these views. The evidence shows the flux of micrometeoroids ($\lesssim 10^{-6}$ g) at the lunar surface has remained in quasi-equilibrium near the present-day value over the past 2×10^9 yr, but that significant increases have occurred. Three near-cyclical enhancements are superimposed on the drill core stratigraphy, with separations of $\sim 10^8$ yr. The magnitudes, durations, and periodicity of the flux increases suggest their origin may be the passage of the Solar System through dust lanes in the galactic spiral arms.

It has been noted⁵ that a segment of the cumulative grain-size distribution of "texturally-mature"⁶ lunar soils closely parallels the present meteoroid flux distribution in the soil grain-size range 44–177 μ m, at the maximum concentration of impact-derived constructional glass particles, or "agglutinates"^{7,8}. We believe that this segment of the soil distribution provides information on the meteoroid flux distribution that produced it.

Most of the kinetic energy in the present meteoroid flux⁹ is in particles of masses $< 10^{-6}$ g which strike the lunar soil at a

velocity of ~ 20 km s⁻¹ and generate enough heat to fuse a volume of soil. Some melt is lost as spray during crater excavation, but morphological information in the form of ring- (Fig. 1) or bowl-shaped agglutinates¹⁰ suggests that only one large agglutinate particle is formed by any one micrometeoroid impact. Thus the parallelism between grain size and meteoroid flux is understandable: one simply mirrors the other. Experimental data on the ratio of mass of the melt to mass of the meteoroid at 20 km s⁻¹ is lacking, but theory⁹ suggests that an agglutinate represents ~ 5 times the mass of the impacting micrometeoroid at 20 km s⁻¹. Using mean agglutinate diameters and a particle density of 3.1 g cm⁻³ the parallel segment of the soil curve converts to a micrometeoroid mass range of 0.08×10^{-6} – 2.0×10^{-6} g.

A meteoroid distribution is $N = am^\beta$, where N is the total number of particles larger than mass m , a is the intercept and β is the slope of a plot of log flux against log mass for masses larger than $\sim 10^{-7}$ g (refs 9 and 11). We convert the bulk grain-size distribution for the 45–177- μ m range from weight % retained in sieves to particle number equivalents assuming spherical particles. The numbers of particles in five size intervals are normalised to the total number, and we construct a cumulative particle number frequency distribution. Using equivalent meteoroid masses and cumulative particle numbers in log-log form, a linear least squares fit gives β . But a (which gives total flux) cannot be found without knowing the meteoroid exposure time of the soil. We derived β for 61 samples—12 surface soils and 49 from the Apollo 15 deep drill core. In all cases the linear fit explains $> 97\%$ of the variance, thus demonstrating a strong linear dependence between log N and log m .

Soils from the lunar surface should give β estimates near the modern flux value derived from other methods, thus offering an independent test of the model. The present meteoroid flux in our mass range has $\beta = -1.213$ (ref. 9). Estimates of β for the surface samples range from -1.266 to -1.142 with a mean of -1.213 ± 0.039 . A one-tailed t test indicates no significant difference between the modern β value and our estimates from

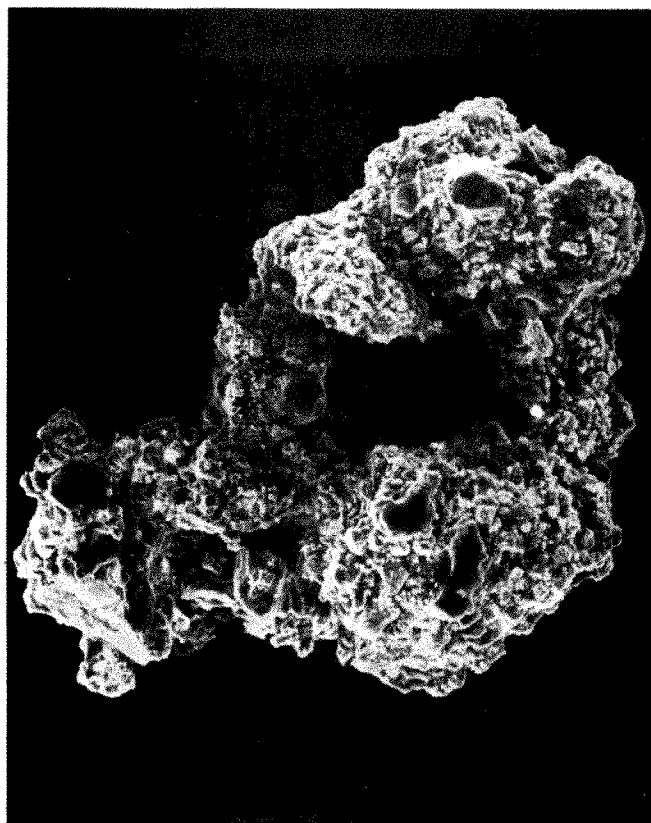


Fig. 1 A ring-shaped agglutinate from the lunar soil. The agglutinate is approximately 180 μ m in diameter.

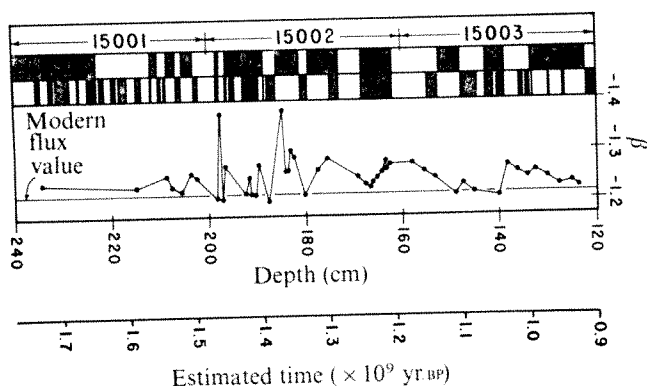


Fig. 2 Estimates of β as a function of depth in the lunar soil. Estimates of β seldom exceed the modern flux value and are not correlated with the soil stratigraphy. The top half of the stratigraphic column shows major units only—the bottom half shows all units identified.

the surface soils ($t_{0.05} = 2.201 > 0.043$, d.f. = 11). That is, the model works.

Large meteoroids produce ejecta blankets of sufficient thickness and extent to survive as layers. This creates three interpretation problems: (1) inverted stratigraphy may appear in the record¹²; (2) old agglutinates may survive excavation from an earlier layer; and (3) the soil accumulation rate is nonlinear^{8,13}.

First, inverted stratigraphic sequences of small thickness will not destroy regular trends in the data, but will increase deviations about the trend. The range in β values is not affected. Second Lindsay⁵ investigated agglutinate survival under reworking and found most pre-existing agglutinates are destroyed in excavation by layer-forming events (that is, crushed to sizes $\lesssim 15 \mu\text{m}$, below our range). Third, soil accumulation rates are nonlinear^{8,13}, but modelling the growth requires assumptions of the meteoroid flux history, so it is best to model linear accumulation. The age of the substrate¹⁴ and total soil thickness at the Apollo 15 site¹⁵ give an average accumulation rate of $1.35 \times 10^{-7} \text{ cm yr}^{-1}$. Nonlinear soil models¹³ fortunately give similar rates for this time period. Since our sampling interval is 0.5 cm our time resolution is $\sim 4 \times 10^6 \text{ yr}$.

The core samples cover a depth of 120–240 cm in known stratigraphy (Fig. 2). Estimates of β for these samples range from -1.199 to -1.381 with a mean of -1.248 ± 0.036 . The data do not show a monotonically decreasing flux distribution but suggest a quasi-steady-state background level near the modern flux value, with enhancements superimposed. Below 180 cm the deviations are a series of disordered 'spikes'; above 180 cm three regular deviations each extend over 15–20 cm of the record. Each cycle crosses several stratigraphic units, indicating that the β variations have extra-lunar, time-dependent sources, rather than being determined by independent depositional processes operating on individual stratigraphic units.

Micrometeoroid experiments on the Pioneer 8, 9, 10 and 11 spacecraft^{18,19} show the asteroid belt is not a small particle source. Unless the particle populations and dynamics in the Solar System were quite different 1–2 eons ago, we suggest that the flux enhancements are not redistributions of matter by asteroidal collisions. We propose the enhancements record passages of the Solar System through dense interstellar clouds, plus active cometary episodes. These two sources may be manifestations of the same event: namely the passage of the Solar System through compression lanes in the arms of the Galaxy¹ and the concomitant generation of comets and their subsequent mass loss to the Solar System²⁰.

Lyttleton^{20,22,25} suggests that comets are "new" members of the Solar System formed by the interaction of the Sun with interstellar clouds. The Sun encounters dense clouds ($\sim 10^{-19} \text{ g cm}^{-3}$) in "compression lanes" of interstellar matter in the galactic spiral arms²⁰. Mass loss from long-period comets so

formed could enhance the small particle flux for 10^7 – 10^8 yr (ref. 17).

Hartung and Störzer¹⁶ suggest the modern small particle flux is increasing due to activity by Comet Encke. A micrometeoroid detector on the Heos-2 spacecraft²¹ shows that Comet Kohoutek deposits small particles into the inner Solar System. Lyttleton²³ suggests that particles $\lesssim 10^{-6} \text{ g}$ are lost from comets due to the solar wind. Since comets have masses of 10^{17} – 10^{20} g and lose $\gtrsim 10^{-4}$ of this in each solar encounter^{20,22}, one comet could increase the particulate material density within the orbit of Jupiter²⁰ by $\sim 3 \times 10^{-24} \text{ g cm}^{-3}$ if the debris is in the ecliptic. For matter collected by the Moon at 20 km s^{-1} , the surface flux would increase by $\sim 10^{-10} \text{ g cm}^{-2} \text{ yr}^{-1}$ above the present level of $1.2 \times 10^{-9} \text{ g cm}^{-2} \text{ yr}^{-1}$ in small particles^{9,11,23,24}. Further flux enhancements are possible for cometary periods which are short compared with the Solar System debris retention time¹⁷. Such enhancements could explain brief flux excursions. The 'peak' at $\sim 200 \text{ cm}$ corresponds to a small particle flux increase of ~ 15 times the present value.

The speed²⁶ of the Sun through compression lanes is 5 – 24 km s^{-1} , so cloud densities as above give enhancements $5 \times 10^{-8} \text{ g cm}^{-2} \text{ yr}^{-1}$. Although interstellar clouds have variable constitution, "typical clouds" contain $\approx 1\%$ grains by mass^{27,28}. For an encounter speed of 20 km s^{-1} , these particles striking the Moon would increase the small particle flux by $\sim 10^{-8} \text{ g cm}^{-2} \text{ yr}^{-1}$. So such encounters are numerically capable of producing the flux changes deduced from lunar soil parameters.

From 120 to 180 cm in the core tube the β variations are smooth, cyclical deviations from the present-day value. Although it is difficult to correlate depth with age exactly, the three cycles have periodicities of 1×10^8 – $2 \times 10^8 \text{ yr}$ and comparable durations. On this basis, the cycles cover an age 0.9×10^9 – $1.5 \times 10^9 \text{ yr}$ (all Precambrian), so unfortunately our data cannot be compared with known glacial epochs. McCrea¹ points out that the Solar System crosses a spiral arm every 10^8 year or so, taking $\sim 10^6 \text{ yr}$ to cross the compression lane and spending a total of $\sim 10^7 \text{ yr}$ in the arm. This agrees well with our estimates of β cycles in the soil, except for their longer durations ($\sim 10^8 \text{ yr}$) which may be due to mass loss to the inner Solar System by long-period comets born in such encounters.

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Jupiter's atmospheric circulation

By numerical integration of conventional meteorological equations at appropriate parameter values we have been able to reproduce most of the major characteristics of the Jovian atmosphere.

The axisymmetry and scale of the bands, the oval-shaped disturbances, the waves and the Great Red Spot are all essentially characteristics of turbulent barotropic vorticity exchanges in a rapidly rotating planetary atmosphere. They are produced by the interaction in a spherical domain of a two-dimensional (horizontal) turbulent cascade and Rossby wave propagation, a process that we shall refer to as global turbulence. This interaction occurs at a length scale $L_B = \pi(2u/\beta)^{1/2}$, where u is a measure of the zonal velocity and β is the northward gradient of the Coriolis force, that closely matches the observed size of the bands. This hypothesis has been verified by solutions obtained for a stochastically forced barotropic equation, see for example Fig. 1.

The complete Jovian thermodynamical system can be reasonably well reproduced by using a standard (that is, Phillips's) terrestrial general circulation model under Jovian parameter conditions (see Fig. 2, for example). Apart from the characteristic banded structure the solutions also reveal the existence of an intra-jet circulation or gyre in which the flow resembles that surrounding the Great Red Spot. The planet also seems to have a heat transfer (index) cycle with a 4 to 5-yr period that accounts for long term variability. Clouds are produced by vertical circulation cells induced by frictional Ekman pumping.

Our theoretical flows suggest that the Great Red Spot can be thought of as essentially the core of an intra-jet circulation or as an eddy of global turbulence (as defined above). Like the ovals,

Fig. 1 An example of simulated Jovian turbulence. Sphere contains stream function contours with negative values shaded by 1/4 of grid points. Profile of longitudinally averaged zonal flow has a scale of 100 m s^{-1} in right-hand side diagram. State is transient and early in atmospheric evolution to final form. Although more orderly than final state it illustrates basic elements more clearly. A cine film of this solution is available on request.

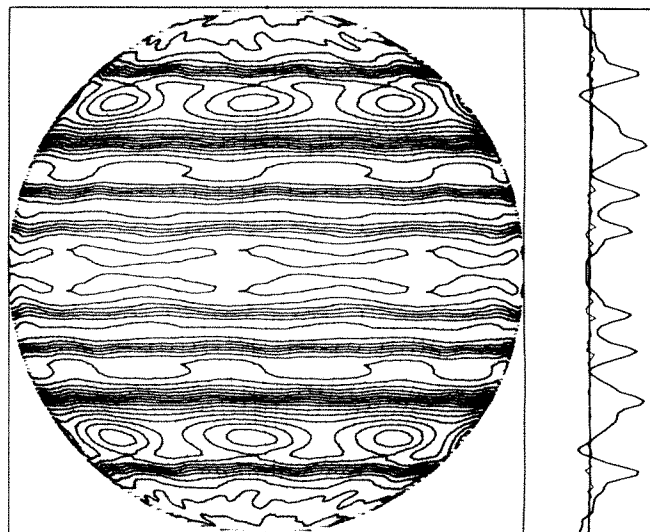
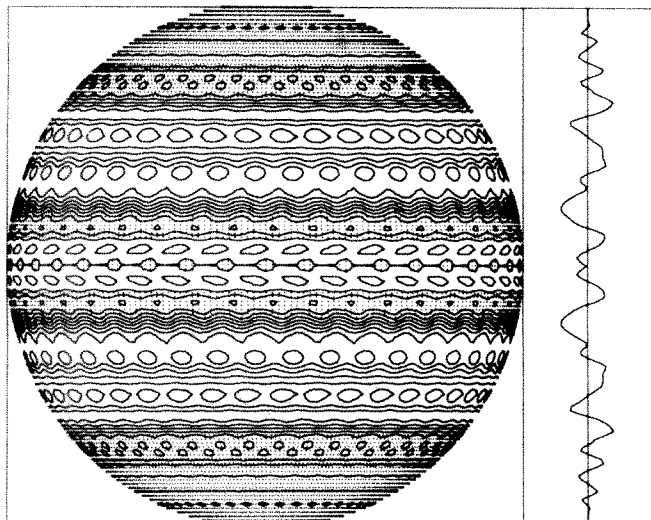


Fig. 2 The global circulation as given by a terrestrial general circulation model integrated in the Jovian parameter range. Calculations were made over a limited sector and repeated for global display. Model is not valid at the equator.

the Great Red Spot plays an important role in the energy cascade that maintains the multiple zonal currents. The persistence of the Great Red Spot is due to the fact that (1) energy cascades toward larger scales in two-dimensional turbulence and (2) under the appropriate conditions large intra-jet circulations such as that forming the Great Red Spot are an integral part of this type of multiple-jet global circulation.

Thus Jupiter's atmosphere seems to have the same dynamical ingredients as the terrestrial atmosphere and ocean. The processes occur on different scales, however, and act in different proportions. As in the terrestrial and Martian systems, baroclinic instability again seems to be the primary energy conversion process. A complete description of the meteorology of Jupiter and Saturn has been submitted for publication elsewhere.

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Noble gases in an Hawaiian xenolith

THE noble gas record in meteorites and lunar samples has been the subject of many investigations aimed at determining their age, the history of their exposure to cosmic rays and to the solar wind, and the early chronology of events in the Solar System (see review in ref. 1). Information on the latter is contained primarily in the isotopes of xenon, where the decay products of extinct ^{129}I and ^{244}Pu provide a record of the synthesis of elements and the early history of planetary solids (see review in ref. 2). The occurrence of radiogenic xenon in CO_2 well gas from Harding County, New Mexico is the only clear evidence that extinct radioactivities were present in the early history of the Earth^{3,4}, but the suggestion that this radiogenic xenon had been brought near the Earth's surface in hot magmas was not confirmed by recent analyses of xenon in lava rock from this region⁵.

The present investigation of noble gases in a volcanic xenolith containing high-purity inclusions of liquid CO_2 was undertaken to see if radiogenic xenon might be associated with

Table 1 Concentrations of noble gases in xenolith

Sample	I	I	Blank I	II	II	Blank II
Weight (g)	21.307	21.307	—	15.136	15.136g	—
Temperature (°C)	600	1,700	1,700	600	1,700	1,700
Concentrations (cm ³ g ⁻¹ at STP)						
⁴ He × 10 ⁸	1.1	32	1.5	1.2	39	1.8
²² Ne × 10 ¹¹	0.52	8.9	2.4	0.56	6.7	1.3
³⁶ Ar × 10 ¹⁰	0.37	4.2	0.31	0.16	3.7	1.1
⁴⁰ Ar × 10 ⁷	0.11	13	0.09	0.47	16	0.31
⁸⁴ Kr × 10 ¹²	0.32	5.8	0.32	0.12	5.6	0.35
¹³⁰ Xe × 10 ¹⁴	0.55	12	0.46	0.34	13	0.40

mantle-derived CO₂ (ref. 6) and to see if the abundance pattern of noble gases within the Earth is represented by the noble gases trapped in glassy margins of certain deep-sea basalts^{7,8}. Two relatively large samples were taken from the interior of a large peridotite xenolith (≈0.5 kg) which had been brought to the surface by the 1801 Kaupulehu flow of the Hualalai volcano, Hawaii. The xenolith is composed of large olivine crystals (≈1–10 mm), which have been shown by density measurements and X-ray analysis to be rich in magnesium, approaching forsterite. The Hawaiian xenoliths are characterised by an abundance of inclusions of liquified CO₂, up to 3% by volume (0.5% by weight)⁹. These inclusions have been shown to contain radiogenic ⁴He and ⁴⁰Ar with a low ⁴He/⁴⁰Ar ratio as might be generated from a source of chondritic composition¹⁰.

To ensure complete autonomy of samples and to guard against the possibility of a systematic error, as might arise from 'memory' effects in the system, the two samples were analysed several months apart. Samples of other material were analysed in the interim period. The samples were mounted in the upper chamber of a water-cooled quartz extraction bottle, the pressure reduced to ≈10⁻⁹ mmHg, and the system blank measured by analysing gases generated in heating the new, empty molybdenum crucible to the highest temperature (≈1,700 °C) to be used in extracting gases from the sample. When the system blank had reached an acceptable level, a system blank for the low extraction temperature (600 °C) was determined, and then the sample was dropped into the crucible for extraction of gases. The gases were collected and analysed at the two extraction temperatures, first at ≈600 °C and then at ≈1,700 °C, at which the sample completely melted. The procedures used in our laboratory for calibration of the mass spectrometer and for extraction, clean-up, and analysis of noble gases have been described previously¹¹.

The concentrations of noble gases found in these samples are presented in Table 1. The gas concentrations were measured by the 'peak height' method, and are estimated to have an error of ±20%, except that ⁴He may have a systematic error due to leakage of helium through our glass vacuum system or through our glass air standards. To compare the abundance pattern of non-radiogenic noble gases in the xenolith with that in other terrestrial materials, we define a fraction factor, f^m , relative to ³⁶Ar,

$$f^m = ({}^m\text{X}/{}^{36}\text{Ar})_{\text{sample}}/({}^m\text{X}/{}^{36}\text{Ar})_{\text{air}} \quad (1)$$

where X represents a non-radiogenic noble gas isotope of mass number, m . For atmospheric noble gases¹² $f^m \equiv 1.00$ at all values of m , and, for all samples, $f^{36} \equiv 1.00$.

Figure 1 compares the values of f^m for noble gases in the xenolith with values of f^m for noble gases in Fig Tree Shale¹³ and in an enstatite melt which equilibrated with atmospheric noble gases¹⁴. The relative abundances of noble gases in Fig Tree Shale show the effect of preferential adsorption of the heavier noble gases^{15,16}, and the gases in the enstatite melt show that a solution of noble gases in melts in equilibrium discriminates against incorporation of the larger atoms. The abundance pattern of Ne, Ar and Kr in the xenolith is very close to that predicted by Kirsten¹⁴ for an enstatite melt which

has equilibrated with a gas reservoir having the relative abundances of Ne, Ar, and Kr that exist in the present atmosphere¹². For Xe the trend is reversed, however, and the xenolith has approximately 10 times more Xe than expected in a melt which has equilibrated with atmospheric noble gases. An apparent excess of Xe in the xenolith can be explained in terms of the selective depletion of atmospheric Xe by adsorption on shales^{13,15,16}. In view of the fact that Hawaiian basalt magma and xenoliths may come from depths as great as 60 km (ref. 6), the abundance pattern of noble gases in the xenolith (Fig. 1) suggests that the relative abundances of Ne, Ar, and Kr in the atmosphere may be a fair representation of noble gases which equilibrated with the mantle, but the Xe/Ar ratio in the mantle may be about an order of magnitude higher than the Xe/Ar ratio in the atmosphere. Thus, the abundance pattern for the total terrestrial inventory of noble gases seems to be very similar to that in typical chondrites, as has been suggested earlier from studies of noble gases on shales^{13,15,16}.

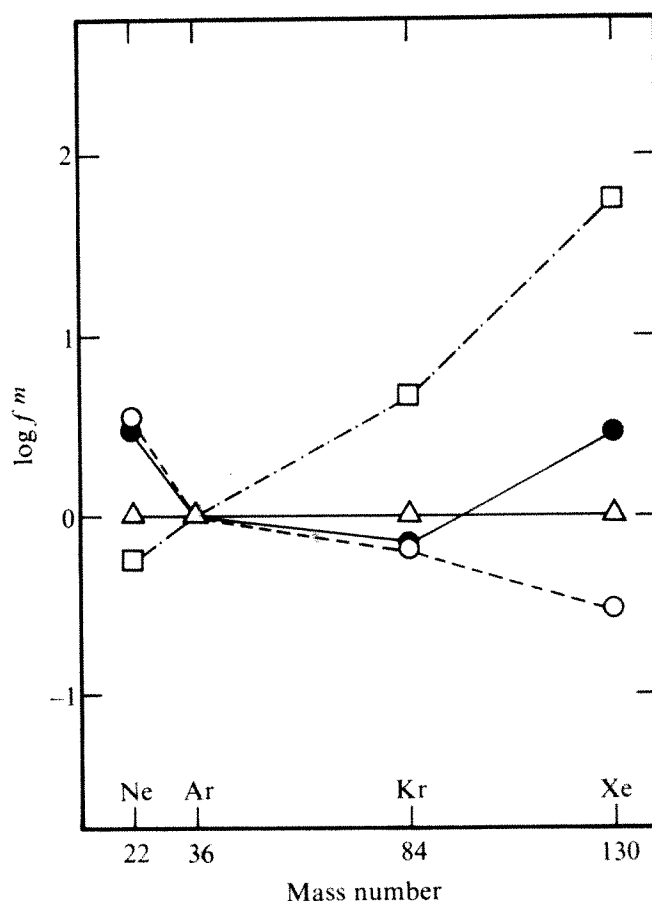


Fig. 1 A comparison of the noble gas abundance pattern in xenolith (●) with that in air (△)¹², in Fig Tree Shale¹³ (□), and in an enstatite melt¹⁴ (○), which has equilibrated with atmospheric noble gases.

The isotopic compositions of the noble gases released from the xenolith at 1,700 °C are given in Table 2, together with the isotopic composition of atmospheric noble gases. Xenon displays a clear excess of ^{129}Xe and a small excess of ^{136}Xe which is too low for a determination of the fission yields. The isotopic composition of the non-radiogenic xenon seems to be atmospheric, and the isotopic compositions of the other noble gases reveal no anomalies, except for radiogenic ^{40}Ar . The relatively high concentrations of ^{40}Ar interfered with measurements of other isotopes of argon and neon. The high beam intensity of $^{40}\text{Ar}^+$ ions caused a rapid build-up of 'memory' peaks at ^{36}Ar and ^{38}Ar , and $^{40}\text{Ar}^{2+}$ ions interfered with the ^{20}Ne measurement. For these reasons the isotopic composition of neon is not reported in Table 2, and only upper limits are given for the $^{38}\text{Ar}/^{36}\text{Ar}$ ratio.

The 4.5% enrichment of ^{129}Xe is greater than that observed in any well gas⁴ except in the CO_2 gas from Harding County, New Mexico, and the ^{129}Xe enrichment here is almost twice as large as that first observed³ in the CO_2 well gas. It should be stressed again that there were no indications during the analyses of memory or contamination at mass number 129, and we are confident that the ^{129}Xe anomaly is real. Possible origins for excess ^{129}Xe in

use of the noble gases in the xenolith to decipher the abundance pattern of noble gases within the Earth.

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Table 2 Isotopic composition of noble gases in xenolith

Sample	I	II	Atmosphere
Weight (g)	21.307	15.136	—
Temperature (°C)	1,700	1,700	—
$^{36}\text{Ar}/^{38}\text{Ar}$	≈ 1.00	≈ 1.00	≈ 1.00
$^{38}\text{Ar}/^{36}\text{Ar}$	≤ 0.212	≤ 0.209	0.187
$^{40}\text{Ar}/^{36}\text{Ar}$	$3,328 \pm 6$	$4,437 \pm 14$	296
$^{80}\text{Kr}/^{84}\text{Kr}$	3.96 ± 0.05	4.04 ± 0.04	3.96
$^{82}\text{Kr}/^{84}\text{Kr}$	20.3 ± 0.1	20.3 ± 0.1	20.2
$^{83}\text{Kr}/^{84}\text{Kr}$	20.2 ± 0.1	20.1 ± 0.1	20.2
$^{84}\text{Kr}/^{84}\text{Kr}$	≈ 100	≈ 100	≈ 100
$^{86}\text{Kr}/^{84}\text{Kr}$	30.6 ± 0.1	30.5 ± 0.1	30.6
$^{124}\text{Xe}/^{130}\text{Xe}$	—	2.38 ± 0.03	2.35
$^{126}\text{Xe}/^{130}\text{Xe}$	—	2.24 ± 0.03	2.21
$^{128}\text{Xe}/^{130}\text{Xe}$	—	47.6 ± 0.4	47.0
$^{129}\text{Xe}/^{130}\text{Xe}$	673 ± 3	677 ± 2	648
$^{130}\text{Xe}/^{130}\text{Xe}$	≈ 100	≈ 100	≈ 100
$^{131}\text{Xe}/^{130}\text{Xe}$	518 ± 2	518 ± 2	519
$^{132}\text{Xe}/^{130}\text{Xe}$	658 ± 3	658 ± 2	659
$^{134}\text{Xe}/^{130}\text{Xe}$	258 ± 2	259 ± 2	256
$^{136}\text{Xe}/^{130}\text{Xe}$	220 ± 2	221 ± 1	217

The isotopic compositions of atmospheric Ar, Kr and Xe are from refs 17, 18 and 19, respectively.

CO_2 well gas have been considered elsewhere³, and the conclusion that this represents the decay product of extinct ^{129}I applies equally well to the xenolith sample. The xenolith sample thus represents the first terrestrial rock in which the decay product of an extinct radioactivity has been identified.

The site of the radiogenic ^{129}Xe in the xenolith was not determined, but the report of radiogenic ^4He and ^{40}Ar in the inclusions of xenoliths¹⁰ and the reports⁴ of radiogenic ^{129}Xe in CO_2 well gas suggest that we may be seeing 'orphan' ^{129}Xe , that is, radiogenic ^{129}Xe from an external reservoir rather than radiogenic ^{129}Xe produced within the xenolith by the decay of ^{129}I . Green⁶ has suggested the possibility of a CO_2 -charged asthenosphere as a source for the CO_2 -rich fluid inclusions in Hawaiian xenoliths. His model implies that the solid Earth is effectively capped against diffusive outgassing by the CO_2 asthenosphere, a result which may account for the terrestrial association of radiogenic ^{129}Xe with CO_2 . The presence of this decay product of ^{129}I ($t_{1/2} = 17 \times 10^6$ yr) in the xenolith is further evidence that the formation of the Earth did not appreciably postdate the formation of meteorites. It also indicates that the xenolith contained juvenile noble gases from the interior of the Earth, and thus lends credence to our

Bottom sliding of a glacier measured from the surface

We have measured the sliding motion at the bottom of a glacier from the surface of the ice. Fleming Glacier in the Antarctic Peninsula is a fast flowing polar glacier, more than 1,000 m thick, feeding the Wordie Ice Shelf. Observations indicate that up to a third of its surface velocity is due to the bottom layers of ice sliding over the bed. Previous measurements of the bottom sliding of glaciers have been confined to tunnels or boreholes that have reached bed-rock^{1,2}; sliding velocities have also been calculated indirectly by using assumptions about the internal deformation of ice³. Results obtained by our method can help to test theories of basal sliding⁴ and should give insight into the mechanism and consequences of surges in glaciers and ice sheets⁵.

The surface velocity can be considered to consist of two components, one representing deformation of ice in the body of the glacier and the other representing basal slip. Conventional optical survey using fixed rock as a reference was used to give the sum of both components of the surface velocity. A radio-echo technique⁶ gave the surface velocity relative to a layer that reflected electromagnetic waves—evidently from a level close to the base of the ice. Since this velocity differed from the survey velocity, it was inferred that the reflecting layer was embedded in the ice. The radio-echo method thus measured only the deformation component of the surface velocity, and the difference between the survey and radio-echo velocities represented the bottom sliding velocity.

Walford⁷ tried the radio-echo technique on Fleming Glacier and concluded that the radio-echo velocity agreed, within his limits of error, with the surface velocity determined by optical survey. We repeated the experiment, at a site within 1 km of Walford's site, in January 1974, but over a longer time to increase accuracy. This time there was a significant difference between the survey and radio-echo velocities⁸. In December 1974, therefore, we investigated

Table 1 Observed surface velocities on Fleming Glacier

	Date 1974	1		2		3	
		m yr ⁻¹	Direction	m yr ⁻¹	Direction	m yr ⁻¹	Direction
Survey velocity	Jan.	201 ± 20	284 ± 5°	175 ± 4	272 ± 5°	146 ± 4	277 ± 5°
	Dec.	201 ± 4	283 ± 5°				
Radio echo velocity	Jan.	138 ± 5	287 ± 1°	113 ± 7	272 ± 1°	139 ± 7	271 ± 1°
	Dec.	142 ± 11	277 ± 1°				
Sliding velocity	Jan.	62 ± 25	284 ± 5°	64 ± 11	272 ± 5°	7 ± 11	277 ± 5°
	Dec.	59 ± 15	283 ± 5°				

Relative velocity between sites 1 and 2 in December 1974 was 29 ± 4 m yr⁻¹ at $272 \pm 1^\circ$. The direction of the sliding velocity has been taken to be that of the survey velocity.

this same site and also two other sites on a flowline upstream from it, to resolve the apparent discrepancy. Figure 1 shows the radio-echo ice thickness profile through the sites occupied in December 1974, and Table 1 gives a summary of the results from the two seasons' work.

The optical survey was carried out by a method of resection. Six permanent reference objects on surrounding mountains were intersected from each end of a baseline 417.45 m long in January 1974, and 5,552.66 m long in December 1974. Subsequent resection of these points gave the movement of each site (J. L. W. Walton, personal communication). A period of about 16 d was necessary between resections to obtain the required accuracy. The change in distance between sites 1 and 2 was measured by a pair of MRA-3 tellurometers, which over a period of 14 d gave the relative velocity between the sites. This agreed with the difference between observed surface velocities at the two sites.

We used a modified Scott Polar Research Institute Mark IV radio-echo sounder to record the bottom-echo fading pattern only. It operated at 35 MHz in January 1974 and at 60 MHz in December 1974. Fading patterns over an area 11×2.5 m were generated by moving the aerial in a series of parallel lines along a track 25 cm above the surface of the snow. The patterns were calibrated with marks representing distance moved from a reference stake fixed in the surface of the glacier. The displacement of successive patterns relative to the stake over a period of days, was measured by comparing the position of distinctive features in the pattern with the distance marks. Values from parallel runs were averaged to give the velocity component along the track; a maximum error of 0.5%, resulting from the difference in angle between the survey velocity and the

alignment of the radio-echo track, can be neglected. Radio-echo velocities were obtained from fading patterns taken over periods of up to 10 d.

Table 1 shows that there is excellent agreement between the January and December results at site 1. Walford's radio-echo velocity at his site (close to our site 1) of 139 ± 11 m yr⁻¹ along $260 \pm 10^\circ$ also agrees with ours, indicating that differences between survey and radio-echo velocities are significant, and due to sliding of the glacier over its bed. Results from site 2 show a similar behaviour, but site 3 seems to have no sliding component. We are not yet in a position to determine whether there really was no sliding at site 3. If sliding did occur but the radio-echo reflecting surface was the actual glacier bed, rather than a layer embedded in the ice near the base, this would account for the anomaly. If the reflecting surface was the true glacier bed it is to be expected that the radio-echo velocity would be the same as the survey velocity.

An ice mass can only slide over its bed if the temperature of the basal ice is at its pressure melting point. Using measured values of the ice temperature at a depth of 10 m (-12.6°C), the observed annual accumulation of snow (100 g cm^{-2}) at site 1 and the surface velocity transformed to an equivalent basal heat flux⁹, steady-state models¹⁰ indicate that the basal ice at site 1 is probably at the melting point. This calculation cannot, however, be sufficiently precise to distinguish between basal ice temperatures at the three sites.

We noticed that the relationships between some features in the fading patterns changed with time. This may be attributed to a gradual deformation of the geometry of the reflecting layer near the base of the ice. Although the nature of the reflecting layer is unknown, it evidently reflects more electromagnetic energy than the bedrock. If the reflecting layer and the bedrock reflected nearly equal amounts of energy, then the received signal, being a combination of echoes from both reflecting surfaces, would be sensitive to relative motion between them. In this case, the fading patterns would not remain recognisable after a period of time. The reflecting layer could be either morainic material², or possibly a layer of ice at its pressure melting point.

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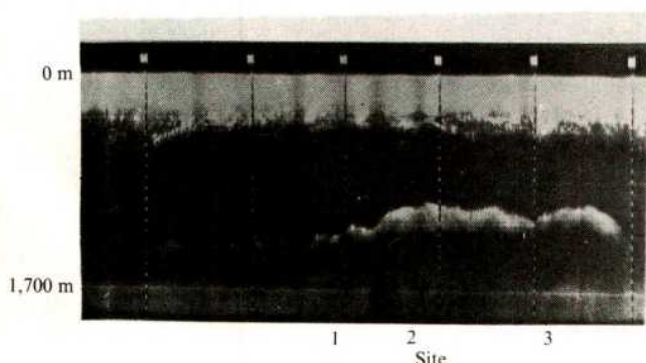
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Fig. 1 Radio-echo ice thickness profile along the probable flowline through the three sites in December 1974. Site 1 was also used in January 1974. The distance between sites 1 and 2 was 5.5 km and between sites 2 and 3 was 3.0 km. The direction of ice flow is from site 3 to site 1.



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An experimental approach to lithification textures

ALTHOUGH observations on changes taking place during the compression of monominerals have been published^{1–3}, little or no information exists on the textural aspects of compression affecting granular sediments. The main reason for this lack of textural information is presumably a technical problem. Ever since the pioneering investigations conducted by Bridgman, equipment used in high pressure experiments in geology has consisted of hydraulic cylinders in combination with completely closed, thin-walled test-tube bombs compressed by the surrounding oil in the hydraulic system^{4,5}. The triaxial cell used in tectonic studies, allowing control over the three stress directions, uses massive samples jacketed to prevent the pressure medium from direct contact with the sample⁴. Triaxial studies have been performed only with massive samples, that is, already lithified rocks. No tests have yet been described involving thin sectioned samples of granular sediments subject to compression. Essentially, such experiments would reveal the mechanical aspects of the burial of natural sediment.

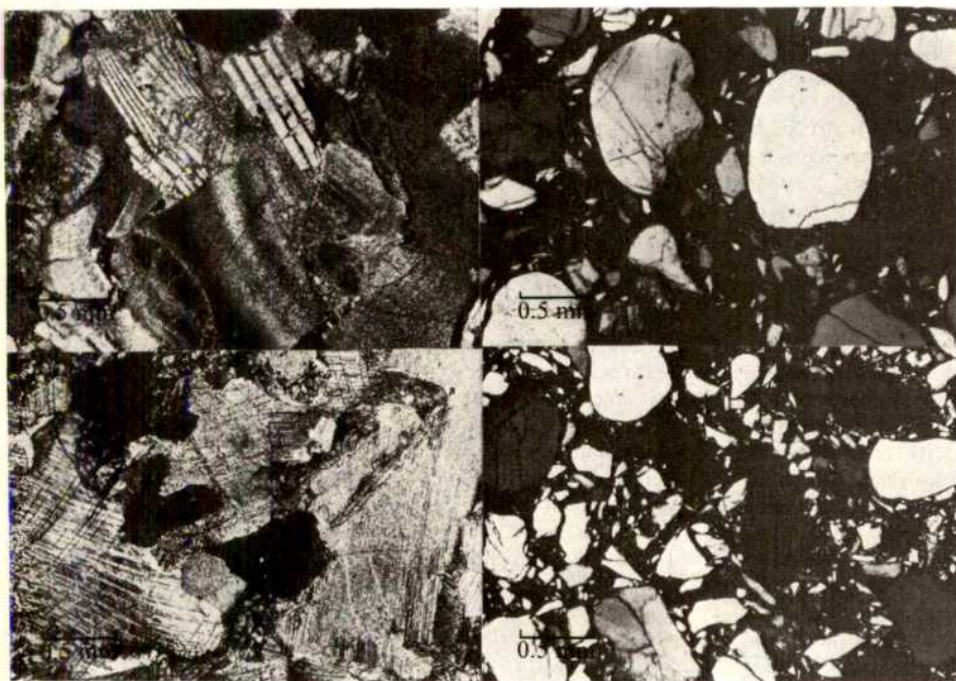


Fig. 1 *a*, Lithification texture produced experimentally during dry compression of various carbonate bioclasts (fragments of clam shells, echinoids, coralline and encrusting algae). *b*, Thin sectioned natural sample (bioclast grainstone). Compare the nature of grain contacts and the absence of porosity with the experimental sample in *a*. *c*, Lithification texture after dry compression of well-rounded quartz grains. Only a few grains remained intact—highly angular fragments and cryptocrystalline quartz powder originate during brittle failure. *d*, Thin sectioned natural quartzite indicating the role of purely mechanical phenomena, such as grain crushing, in the creation of lithification textures in natural sediments.

As a first step in the experimental investigation of lithification textures, brass pressure cells were constructed. A massive steel piston was used to create a confining pressure in brass rings (20 mm high, with walls 2 mm thick). A first series of experiments, consisting of the compression of various dry granular sediments, revealed that a considerable degree of grain crushing took place. With granular material, shear stresses at grain contacts predominate during compression, initiating brittle failure. Compression of only a few grains in excess liquid medium, creates a hydrostatic pressure, without markedly affecting the grains. Thus, by varying the initial quantity of pore liquid mixed with the dry sample, control over pore pressure can be obtained.

The pore medium used in the experiments consisted of a liquid plastic. Minerals are unlikely to dissolve in, or react chemically with, this, and the plastic acts at the same time as the embedding medium, allowing thin sectioning of the compressed sample. After mixing a small quantity of dry granular sediment (for example, quartz sand, carbonate bioclasts, calcite crystals, fragments of feldspar, garnet, topaz, beryl) with different amounts of the liquid plastic, compression took place, reaching maximum loading in minutes. Samples were compressed with a load of 7,000 kg, creating confining pressure of some $1,000 \text{ kg cm}^{-2}$. This value of 1 kbar corresponds to a depth of burial of some 4.7 km when using an average density of the sediment cover of 2.7 g cm^{-3} . No means of artificial heating was used; therefore, the textural changes are caused by mechanical processes only (see also ref. 6). After hardening of the plastic, the brass ring was partially removed using a rotary cutter. After thin sectioning, samples were analysed with a polarising microscope.

Examination of numerous samples that were subjected to laboratory compaction reveals the existence of two major mechanisms responsible for the creation of lithification textures: plastic deformation and grain crushing. Two, more or less characteristic examples of the newly created lithification textures were selected (Fig. 1*a* and *b*). Rapid compression of a quantity of calcium carbonate bioclasts (clam shells, echinoid fragments, coralline algae, and encrusting algae) to $1,000 \text{ kg cm}^{-2}$ not only leads to the total disappearance of porosity (and permeability), but also causes the creation of a multitude of indentation contacts resulting from the plastic deformation of the grain contacts

(Fig. 1). Although a certain degree of grain crushing (brittle failure) takes place during the compression of the bioclasts, almost all the grains still show clear evidence of their original texture. The nature of grain contacts formed should be compared with thin-sectioned natural samples to evaluate the possible role of plastic deformation in the creation of lithification textures. As can be seen in Fig. 1*b*, a thin section prepared from a natural bioclast grainstone, indentation grain contacts and the total absence of porosity are also characteristics of certain natural limestones.

The second mechanism responsible for the creation of lithification textures is observed on dry compression of a well rounded quartz sand at a confining pressure of $1,000 \text{ kg cm}^{-2}$. Many of the well rounded grains break into

angular fragments and even into smaller fragments closely resembling cryptocrystalline quartz (Fig. 1c). Comparison with a natural quartzite sample (Fig. 1d) reveals once again the value of the experimental creation of lithification textures: the microfabric formed closely resembles that of the sample obtained by laboratory compression. The experiments reveal the existence of mechanisms other than the geochemical reactions postulated under the concept of "diagenesis".

In conclusion, the significance of the experimental approach in the analysis of lithification textures seems to have been established, adding an extra dimension to existing methods of geological high-pressure research.

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Stable carbon and oxygen isotope ratios of groundwaters from the Chalk and Lincolnshire Limestone

SAMPLES of groundwater have been collected from the Chalk of the London Basin and the Lincolnshire Limestone in eastern England to determine the radiocarbon and the $^{13}\text{C}/^{12}\text{C}$ ratio in the dissolved carbonate species, and the $^{18}\text{O}/^{16}\text{O}$ and D/H ratios of the water. The results will be published more fully elsewhere, but here we report preliminary findings of the $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios and their implications.

In the London Basin the $\delta^{13}\text{C}$ changes from about -13‰ in and near the outcrop of the Chalk to values less negative than -1‰ about 8 km from the outcrop; the least negative value recorded was -0.5‰ (Fig. 1). Similarly, in the Lincolnshire Limestone, values became less negative in the direction of groundwater flow, reaching -2‰ some 15 km

from the outcrop. These low negative values are unusual. They approach those for the carbon of the London Chalk and Lincolnshire Limestone matrices of $+2.4\text{‰}$.

The radiocarbon content of carbonate species dissolved in groundwater is a function of: (1) the time that has elapsed since the carbonate was in contact with a source of modern carbon, and (2) reactions between the aquifer and the dissolved carbonate species.

The latter have an important bearing on the interpretation of the radiocarbon results. The $^{13}\text{C}/^{12}\text{C}$ ratio is essential for the evaluation of the magnitude of reactions, other than radioactive decay, which may change the ^{14}C content of the groundwater¹. The use of the $^{13}\text{C}/^{12}\text{C}$ ratio in the interpretation of ^{14}C data is based on the fact that the principal sources of the carbonate species in water give different values for the ratio. Thus, either the contribution of different species to the measured ^{14}C in the sample can be estimated, or the measurements can assist in the understanding of the groundwater chemistry. Interpretation of the carbonate chemistry is necessary to assess the correction factor that must be applied to the radiocarbon content because of dilution by non-radioactive carbon.

Although changes in the carbonate chemistry of groundwater in the London Chalk and Lincolnshire Limestone do take place in a down-gradient direction (because of ion exchange, carbonate precipitation and sulphate reduction^{2–4}), these changes alone cannot account for the very small negative values measured. The bicarbonate concentration of the water only increases from about 270 mg l^{-1} in the Chalk outcrop to about 380 mg l^{-1} in the centre of the London Basin (a distance of some 15 km). Similarly, in the Lincolnshire Limestone the increase is only from 270 mg l^{-1} in the outcrop zone to about 460 mg l^{-1} down gradient.

In view of this, the low negative values seem to be due to either isotopic exchange between the carbonate in the aquifer and bicarbonate in the water or continuous precipitation and solution of carbonate as the water flows slowly between the individual constituent particles of the limestone matrix. This view is supported by the fine-grained nature of the aquifers, particularly the Chalk, and the consequent large surface area that exists in contact with the water. Isotopic exchange is, however, a very slow process and is unlikely to have had a significant effect over the timespan during which radiocarbon dating can be applied¹. Therefore continuous precipitation and solution is favoured as the process responsible for the low negative $\delta^{13}\text{C}$ values.

On the basis of this interpretation, the radiocarbon analyses have been corrected to derive an age for the water

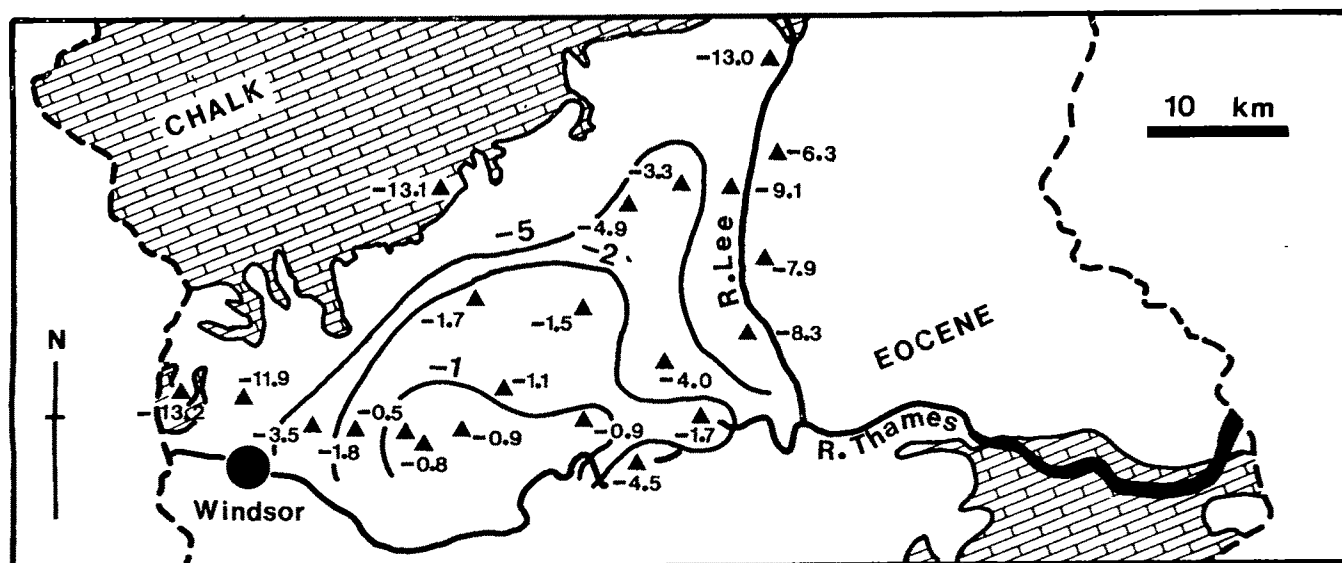


Fig. 1 $\delta^{13}\text{C}$ values (parts per 10^3) for groundwater in the Chalk in part of the London Basin.

in the centre of the London Basin and in the eastern part of the Lincolnshire Limestone in excess of 25,000 yr. The correction assumes that in fine-grained carbonate aquifers, the volume of the rock greatly exceeds the carbonate precipitate, and re-solution of previously precipitated carbonate is insignificant.

An age greater than 25,000 yr implies that the water originated in rainfall during the Pleistocene. The ratios of the stable isotopes $^{18}\text{O}/^{16}\text{O}$ and D/H in this Pleistocene water are in the proportions found in present-day normal meteoric water¹. The range of $\delta^{18}\text{O}$ in the Pleistocene water in the centre of the London Basin is from -7.7% to -7.9% whereas in modern water from the periphery of the Basin, the range is -7.1% to -7.2% . This suggests that either the temperatures at the time of recharge were less than one degree cooler than today², or the weather patterns producing the recharging rainfall differed from those operating now. A possible explanation is that recharge occurred during relatively mild climatic phases of the Weichselian glaciation and that it was limited to summer periods because frozen ground prevented recharge in the winter.

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Processing structure of sentence perception

To extract meaning from a sentence, the listener must combine information from several levels of linguistic analysis. Psycholinguistic research¹ has documented a range of processing levels from the phonetic to the semantic, but has not explored the central problem of specifying the interactions between these levels. We have examined three fundamental questions about the processing structure of sentence perception. When, during sentence processing, does information at different levels become available to the listener; what is the time-course of the interactions between these levels; and what is the direction of information flow between them?

We used three speech monitoring tasks, in which the subject listens to a sentence and makes a timed response when he hears the target word. Identical monitoring, in which the subject knows in advance exactly which word to listen for, provides a baseline for assessing performance in the other two tasks. In rhyme monitoring, where the subject listens for a word that rhymes with the cue word given in advance, his response need only require a phonetic analysis of the target. But in category monitoring, where the target is a word belonging to a taxonomic category specified in advance, the response depends on a semantic analysis of the target. By placing these target words at different serial positions in the test sentences, we can measure the time course of different levels of processing.

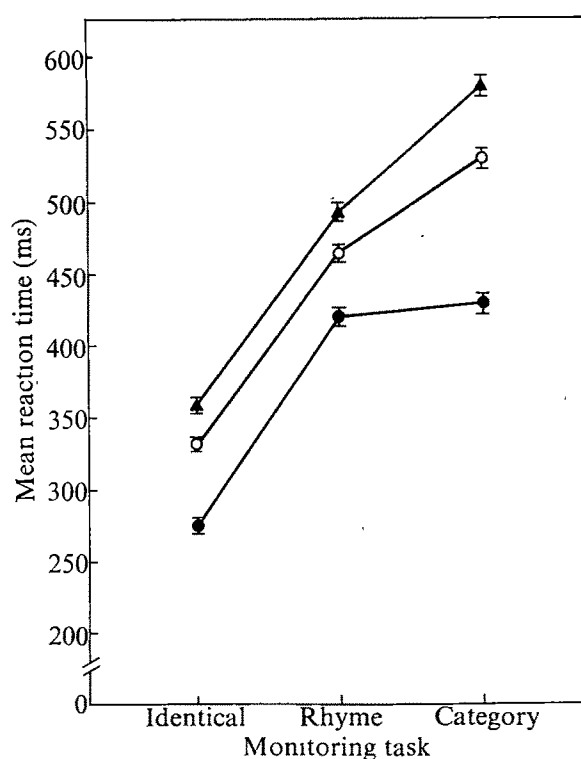
The three monitoring tasks were also co-varied with three

types of prose context—normal prose (NP), syntactic prose (SP), and random word order (RWO). If monitoring in an NP context is facilitated by the presence of sentence-level syntactic and semantic information, then response latencies will increase when there is no semantic interpretation available (in SP), and when syntactic information is also unavailable (in RWO).

Our earlier research^{2,3} leads us to propose an interactive parallel model of sentence processing, in which, from the first word of a normal sentence, the analysis of the input is conducted at all available processing levels. In particular, the information available at any one level of analysis can constrain and facilitate decisions at any other level, so that the continuing phonetic and lexical processing of each word is directly influenced by its current syntactic and semantic context³. As we will make clear in discussing the results, the predictions of this approach will contrast with those of serial theories, which assume varying degrees of delay before information at any one level of analysis can interact with information at any higher level^{1,4}.

The experiment, in summary, combines three monitoring tasks, three prose contexts, and nine word positions. Eighty-one target words were distributed equally across the second to the tenth word positions in the second sentence of 81 pairs of NP sentences. The same target words in the same word positions were also embedded in 81 SP and 81 RWO sentences. These materials were then assigned to nine versions, such that each of 45 subjects heard each of

Fig. 1 Overall monitoring response latencies (with standard errors indicated) for each monitoring by context condition. Each cell represents the mean of 405 observations, counterbalanced so that each subject, target word and word position contributed equally to each of the nine means. The SP sentences were constructed by replacing pseudo-randomly all content words in the NP sentences, except the target words, with new words of the same form-class and frequency. The RWO sentences were constructed by scrambling the word order of the SP sentences, keeping the serial position of the target word constant. The same target words occurred in each monitoring condition, but with different cue words given in advance. Thus, if the target word was 'cat', the cue word for identical monitoring was also 'cat', while in rhyme monitoring it was 'pat', and in category monitoring the category name 'animal'. ●, NP; ○, SP; ▲, RWO.



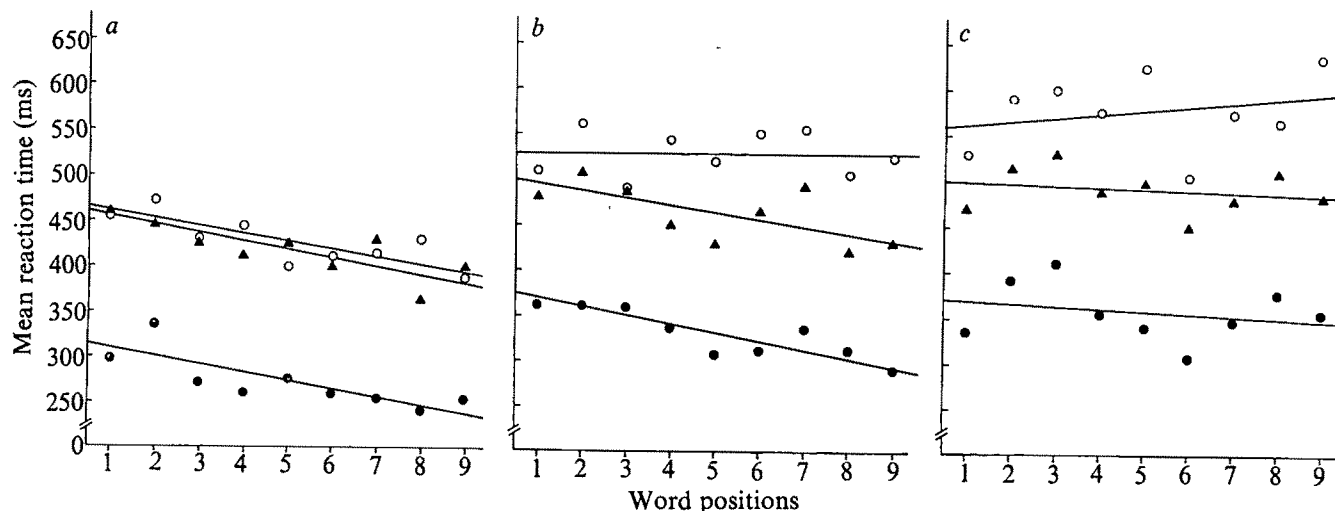


Fig. 2 Monitoring response latencies in each monitoring by context condition as a function of the word-position of the target word. Each point is the mean of 45 observations. Best-fitting least squares regression lines are drawn for each condition. Word-position 1 is the second word in the test sentence. *a*, NP; *b*, SP; *c*, RWO; ○, category; ▲, rhyme; ●, identical.

the 81 target words in one of the nine combinations of context by monitoring conditions.

The overall results are summarised in Fig. 1. Min F^1 values were computed⁵, using the untransformed raw data, with missing observations (less than 2%) replaced. The main effects of monitoring and of context, and the interaction between them, were significant ($P < 0.001$). Each of the means differed significantly from all other means ($P < 0.01$), with the exception of NP rhyme and category monitoring.

Monitoring latencies are faster in all NP conditions than in SP or RWO, and SP monitoring is similarly superior to RWO. This is predicted by the interactive parallel model, in which both semantic and syntactic information interact directly with continuing lower level decisions. On a serial model, lower level analyses are performed first, before being integrated with higher level information. Thus there should be no latency differences between contexts when the monitoring response could be based just on lower-level information—as in rhyme monitoring.

In addition, there are no differences between rhyme and category monitoring in an NP context, but large differences in SP and RWO. According to an interactive parallel model, semantically based decisions in NP can be just as fast as phonetically or lexically based decisions, since the listener develops a unified multi-level representation of the material as he hears it, and the higher and lower level attributes of this representation are equally accessible for making the monitoring response.

In contrast, any serial model has to predict longer latencies for category monitoring, since the defining characteristic of a serial model is that higher level decisions must, to some measurable extent, await lower level decisions. It should be noted that category monitoring in SP and RWO is much slower than rhyme monitoring, showing that the similarity between them in NP depends on the continuing semantic interpretation at the sentential level.

Effects of word position (Fig. 2) are found only in the conditions where hearing more of the sentence gives the subject additional information at the higher levels of analysis relevant to his responses. Latencies decrease as a linear function of word position in all NP monitoring tasks and in SP identical monitoring, with a weaker effect in SP rhyme. In particular, the goodness of fit and the slopes of the linear regression lines are remarkably similar for these major effects (Table 1).

Thus the advantage of NP over SP in identical monitoring is the same at all word positions. Similarly, within NP, the dissociation between identical and rhyme or category

Table 1 Regression of context by monitoring latencies on word position

Monitoring task	Prose context		
	NP	SP	RWO
Identical	$r = -0.78^*$ $b = -8.20$	-0.87^* -8.62	-0.21 -2.35
Rhyme	$r = -0.79^*$ $b = -8.18$	-0.64^\dagger -7.27	-0.19 -1.68
Category	$r = -0.78^*$ $b = -7.67$	-0.005 -0.05	$+0.25$ $+3.78$

r , Pearson product-moment correlation coefficient; b , slope of linear regression line in ms.

* $P < 0.001$.

† $P < 0.05$.

monitoring is the same over word positions. This bears out the interactive parallel claim that analyses at all levels are initiated from the first word in a sentence. Although a serial model could predict differential decreases in latency for the later word positions, the differences between conditions should be minimal earlier in the sentence.

The data, therefore, consistently support an interactive parallel model. On the assumption that the monitoring task reflects the processes involved in ordinary listening, we conclude that a representation of the input at all available levels is initiated immediately in sentence processing, such that bi-directional and reciprocal interactions between these levels also begin very early in processing. The processing of spoken language, whether by humans or computers^{6,7}, may only be feasible when all available constraints, at whatever levels of analysis, are brought together during processing to limit the size of the search space within which the interpretation of the input is being established.

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Determination of perspective reversals

MANY outline drawings of geometrical objects go through apparent reversals of perspective during prolonged viewing, and no adequate explanation for this is known¹. A classical explanation is that satiation or fatigue of the neural process which mediates the perception of one perspective leads to a replacement of the process by another one which has not been recently active²⁻⁴. I tested the satiation theory by studying whether the apparent reversals of perspective can be determined by adaptation to an unambiguous perspective seen in a stereogram or in a three-dimensional object. Adaptation blocked temporarily the appearance of the corresponding perspective in a reversible perspective figure.

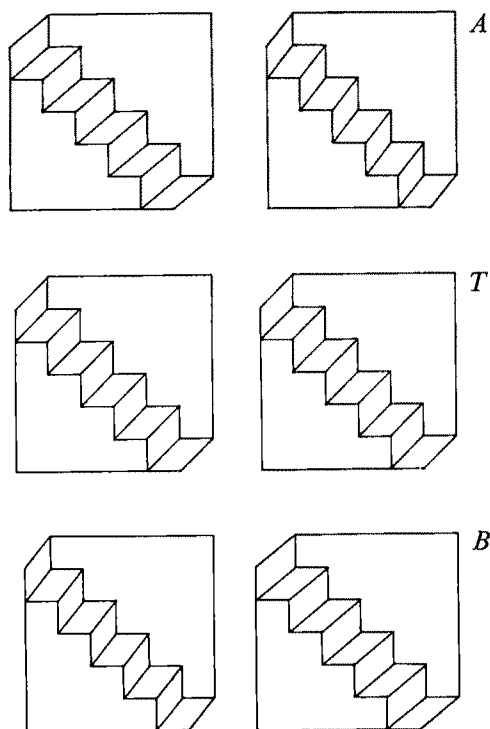


Fig. 1 Determination of perspective reversals through disparity specific adaptation. *A* and *B*, Adaptation stereograms with opposite disparities. *T*, Test stereogram without disparity. If reader stereoscopically fuses the test pair, an alternating perspective of the staircase is seen. Pairs *A* and *B* if properly fused produce a stereoisimage which does not alternate in perspective. Adapt to *A* for a minute and observe then *T*: the apparent perspective of the test image now is opposite to that observed in *A*. Repeat the same by adaptation to *B* and notice that after adaptation to *A* or *B* the perspective of *T* is always opposite to the one observed in the adaptation figure.

The Schröder staircase served as the stimulus figure. The adaptation and test stereograms are shown in Fig. 1. There was no disparity in the test stereogram *T*. So its apparent perspective alternated: the staircase could be seen either from above (case *A*) or from below (case *B*). The adaptation stereograms (*A* and *B*) contained 3 mm of horizontal disparity so that pair *A* formed a stereoisimage in which the staircase was seen from above and pair *B* formed an image in which the staircase was seen from below.

The stereograms were projected on to a screen with the aid of two timer-controlled projectors behind the screen. The subject viewed the screen at a distance of 57 cm, with his head held against a chin rest. A black divider extended

from the subject's forehead to the screen; hence, each eye saw only one half of the tangent screen. One half of the stereogram was shown to each eye. Prisms and lenses fitted into a spectacle frame corrected the retinal images of the figures so that a good fused image was formed. Subjects who were not able to form a stable stereoisimage were rejected.

Black fixation crosses, one for each eye, were continuously present on the screen. With adequate fusion, the subject saw one luminous staircase figure and one black fixation cross in its centre. The fixation crosses were 6.4 cm apart and the size of the fused figure was 5.2 × 5.2 cm. The luminance of the stimulus lines was about 500 cd m⁻² and that of the background was 50 cd m⁻².

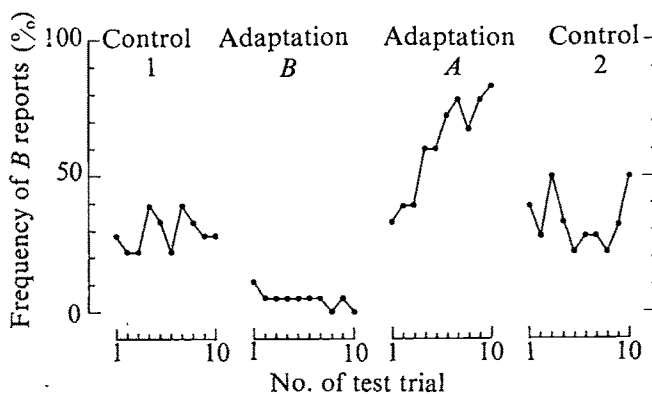
Four adaptation series were run, each consisting of ten trials. Each trial consisted of four events: (1) 5-s fixation to the adaptation figure, (2) a half second pause during which fixation was held, (3) one second fixation to the test figure, and (4) 10-s pause. The subject reported verbally or by means of a switch the apparent orientation of the staircase during its presentation. The test stimulus itself was used as the adaptation stimulus in the first and last adaptation series (controls 1 and 2). In the middle series, pairs *A* and *B* were used as adaptation stereograms, in alternating orders for different subjects.

Figure 2 shows the results for 18 subjects. When a stereogram without disparity served as the adaptation stimulus, 29.4% of all reports in control 1 and 33.3% in control 2 were case *B* reports. After adaptation to type *B* stereogram the average number of *B* reports fell to 4.6% and after adaptation to type *A* stereogram the number rose to 61.1%. Thus, it is clear that adaptation to a stimulus containing disparity led to a considerable decrease of reports of similar perspective in the test stimulus. The effect became stronger as a function of repeated adaptation. At the end of the ten-trial series 83% of subjects reported case *B* in the test figure after adaptation to *A* and no subject reported *B* after adaptation to *B*. Thus, 50 s of distributed adaptation time led to an almost complete determination of the perspective perceived in the neutral test figure.

The decay time of the adaptation effect was determined for 6 subjects by recording the apparent perspective of the test stimulus continuously. After a 1-min adaptation to a stereogram with disparity the average frequency of *B* reports returned to the unadapted level in about 20 s.

In some experiments, a three-dimensional Schröder staircase model was used as the adaptation stimulus. The

Fig. 2 Development of the disparity specific aftereffect during repeated testing after various forms of adaptation. Ordinate shows the percentage of subjects ($N = 18$) who saw perspective alternative *B* in the test figure which could be seen either as alternative *A* or alternative *B* during its 1-s presentation. Each test trial was preceded by a 5-s adaptation period. In control series the adaptation stimulus was the same as the test stimulus. In adaptation *B* the disparity of the adaptation stimulus evoked a perspective view of type *B*. Adaptation stimulus *A* evoked perspective *A*, respectively.



model was made of plastics and the edges of the model were black; the model produced projections like the ones shown in Fig. 1. Adaptation to the model prevented the visibility of the adapted perspective in a corresponding two-dimensional projection of the model. Thus, adaptation to a three-dimensional model produced the same result as adaptation to a stereogram.

These aftereffects are similar to other aftereffects in the third dimension⁵⁻⁷ in the sense that they are generated through adaptation to binocular disparity. They differ in the test stimulus in which the effects are observed. In the usual disparity-specific aftereffects the perceived depth of the test stimulus depends on actual disparity and the effect of adaptation can easily be understood through a change of perceived disparity. The present aftereffects are observed in the perspective of an object which is represented by a drawing; the apparent depth of the depicted object is not conveyed by means of disparity cues. The effect of adaptation in this case is paradoxical because adaptation to disparity affects a perceptual property which is not presented through disparity.

The results support the satiation theory of perspective reversals because they show that forced adaptation to one perspective view decreases the likelihood of continued perception of the same perspective. The role of binocular disparity in the adaptation effect is puzzling, however. It is evident that the satiation theory is not strictly testable as long as the neural processes mediating the impression of perspective in an object presented by a two-dimensional outline drawing remain unknown. The present results indicate that satiation taking place in disparity-specific neural mechanisms⁸⁻⁹ would be sufficient for inducing perspective reversals. The state of disparity-specific neural mechanisms seems to be one possible determinant of apparent perspective seen in drawings but the results do not imply that the perspective reversals ordinarily seen are caused by adaptation of these neurones.

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Is the aestivating lungfish the first vertebrate with suctional breathing?

THE current view is that ventilation of the lungs by aspiratory, negative-pressure inhalation probably occurred in the earliest, now extinct amphibians¹. But no aspiration breathing has been demonstrated in extant air-breathing vertebrates below reptiles, which use freely movable ribs and specialised muscles for this purpose. We report experiments showing that the aestivating lungfish relies on suctional breathing.

When the lungfish is in water the lung is ventilated by essentially aquatic breathing movements involving a buccal, positive-pressure swallowing of air, whereas expiration is largely effected by passive compression of the lung from the external water as the head is raised to the surface and the glottis opened². This basic mechanism of inspiration was passed on in only slightly modified form to the lunged amphibians. When the lungfish aestivates, however, in a soil-encased cocoon³, conditions for breathing are quite different. Air breathing alone can support its aerobic metabolism

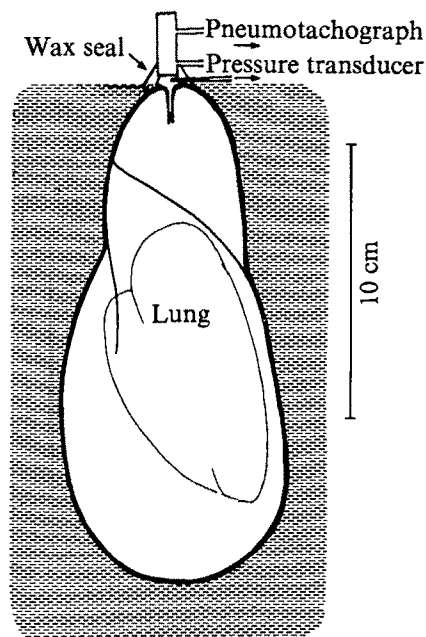


Fig. 1 Schematic representation of aestivating lungfish showing position of pneumotachograph tube and cannula for pressure measurements. The fish is covered by a cocoon (solid outline) and embedded in soil.

when, at the start of aestivation, the lungfish becomes enveloped by a hardened mucous secretion. This cocoon covers the entire surface and even enters the mouth to form a hollow, non-collapsible breathing tube connecting the bucco-pharyngeal cavity with the air channel in the soil leading to the outside air. This prevents the movements of the buccal floor, such as practised in the free swimming lungfish. More importantly, it prevents complete closure of the mouth and thus compels the animal to suspend positive-pressure buccal pumping for filling its lung.

The lungfish, *Protopterus amphibius*, Trewavas, used in our study were naturally aestivating and had been dug out of a dried up lake bed near Malindi, Kenya, 2 yr previously. Eight fish encased in the same soil in which they had entered aestivation, were transported by air to Aarhus where they were kept at 25 °C for 18 months before our study.

The breathing pattern of the fish was recorded continuously by pneumotachography (Statham-Godart) and pressure measurements (Statham) (Fig. 1). In addition, the soil surrounding some of the aestivating lungfish was removed carefully to expose the head and floor of the buccal cavity for visual observation of movements correlated with breathing. In one specimen a catheter was passed through the hollow breathing tube and into the buccal cavity to record pressure. Finally, one aestivating specimen was subjected to X-ray examination to reveal the size of the lungs and airways.

Figure 2 shows that normal breathing was periodic with a series of ventilatory movements alternating with apnoeic periods, which lasted from a few minutes to several hours. A ventilatory period always started with an inspiration. Inspiration was a two-phasic process—an initial phase of relatively rapid inflow (0.2 ml s^{-1}) ran, after a period of no air flow, into a second inspiratory phase when volume flow was less (0.1 ml s^{-1}) but lasted longer (Fig. 2). The longer lasting phase contributed most to the inspiratory volume, which typically totalled about 0.3 ml. The inspiratory phases lasted about 70% of the entire breathing cycle. The two phases of inspiration were attended by two cycles of negative (subambient) pressure recorded in front of the breathing tube. The second inspiratory phase ran directly into a single-phase expiration which was the most forceful of the breathing movements, resulting in air flows exceeding 1 ml s^{-1} .

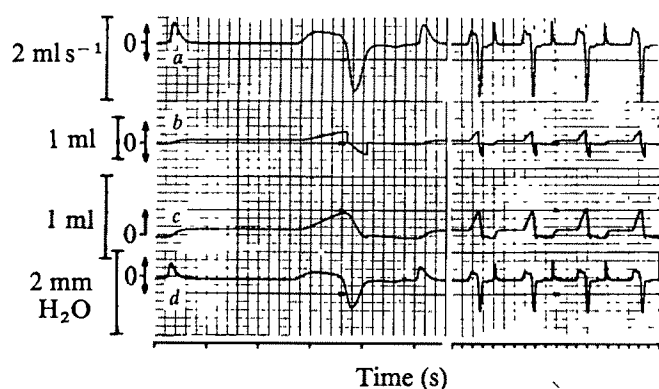


Fig. 2 Tracings showing: a, rate of inspired (upward deflection) and expired air flow, \dot{V} ; b, inspired and expired volumes V_{I+E} ; c, inspired volume, V_I ; d, pressure at entrance of breathing tube, P .

A positive pressure (0.3 mm H₂O) more than twice the value of the negative pressure associated with inspiration correlated with the rapid expiration lasting about 10% of the breath cycle. The expired volume matched the combined volumes of inspiration in phases one and two. Thus both volumes inspired enter the lung and must do so as a result of suctional inhalation.

Pressure pulses recorded in the buccal cavity closely resembled those recorded in the air space in front of the breathing tube (Fig. 1). This is further evidence that air enters the lung by suctional attraction. If the first volume of air entering the lungfish had been positioned in the mouth for a second phase positive-pressure force filling of the lung, two positive-pressure phases, one attending the buccal force pump, the other the expiratory phase, should have been recorded from the mouth catheter. Further, if a positive buccal pressure filled the lung, no air flow should have been recorded through the pneumotach. As the recordings show, all pressure pulses were associated with air flow through the head of the pneumotach. Visual observation of the buccal floor during breathing showed no movements suggesting filling or emptying of the buccal cavity.

The X-ray study similarly showed a rather narrow air channel extending from the breathing tube through the buccopharynx to the lung. A buccal force filling the lung is thus also ruled out on this basis. The X rays revealed a surprisingly large lung shadow. A large lung volume in the aestivating lungfish was also suggested by the observation⁴ that a carefully excavated aestivating lungfish would float high when returned to water. Conversely, the breathing volumes recorded presently from aestivating lungfish are much smaller than those earlier estimated indirectly from non-aestivating fish⁵.

These data tend to support the hypothesis that suctional breathing probably evolved first in aestivating lungfishes. The mechanics of the breathing process and the activity of the associated muscles and skeletal parts are unknown.

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Chemically-mediated host selection in a parasitic mite

MITES parasitise a broad spectrum of metazoans—molluscs, arthropods and vertebrates, including man. They transmit various pathogens to these hosts, and may cause severe medical and economic damage^{1,2}. Host preferences are commonly restricted to one or a few species³, and parasitism may result in profound ecological or biological effects, for example, the regulation of clutch size in birds⁴. Such effects have prompted some investigators to suggest the use of acarine parasites as natural control agents^{5,6}. In spite of all these factors, little is known about how mites select their hosts. We studied host discrimination in a typical acarine parasite, *Proctolaelaps nauphoetae* (Womersley)^{7,8}, and report that it uses a chemical sense to guide it to its only host, the cockroach *Nauphoeta cinerea* (Olivier).

Studies of other arthropod parasites (Hymenoptera) view host discrimination as three consecutive processes⁹⁻¹²: finding the host's environment, selecting an appropriate host, and accepting or parasitising this choice. Acarine parasitism seems to fit this pattern. In this report we focus on the mechanisms by which *P. nauphoetae* selects its host.

Mites use their first pair of legs as chemosensory organs^{1,2}. Ablation of these appendages renders *P. nauphoetae* incapable of finding a host¹³. Therefore we examined the attractiveness of potential chemical stimuli in two-choice preference tests. Material was applied to 7-mm paper disks and exposed to 15–25 mites in a specially designed chamber¹³. Each pair of stimuli in a test was matched in shape, size, texture, solvent and treatment so that the only difference between them was the material being tested. Mite distributions were determined after 3 h, and each experiment was replicated five or more times. The null hypothesis in all experiments was that mites would be equally distributed between the two choices. The G statistic was used to test the homogeneity of replicates and their goodness-of-fit to the null hypothesis. Probabilities cited below refer to the goodness-of-fit. Control experiments showed that mite movements were not influenced by chamber design ($P > 0.9$) or the presence of other mites ($P > 0.9$). Mites avoided white light ($P < 0.001$) but not red ($P > 0.9$); therefore all experiments were conducted under red illumination at constant temperature (27 °C) and humidity (50%). More than 12,000 mites were tested.

N. cinerea is a gregarious cockroach, forming colonies of several hundred individuals which live in close contact with one another and their own detritus¹⁴. As a result materials from faeces, expectorants, dead individuals and rotting debris are carried by each cockroach and could be used by mites as cues. We collected faeces from nine species representative of all phylogenetic lines of cockroaches¹⁵. Mites were attracted to all these ($P < 0.05$) except one, *Eurycotis floridana* (Walker), known to produce a strongly repellent compound¹⁶. Faeces from a laboratory rat were also attractive ($P < 0.01$), as was skatole ($P < 0.03$). These results suggest that mites are generally attracted to faeces, but do not rule out the possibility that additional attractive materials occur in host excrement. This was shown not to be the case: in a choice between host and non-host faeces, mites were equally attracted to both ($P > 0.95$).

In addition to faeces, mites showed a nonspecific attraction to legs excised from several cockroach species ($P < 0.01$), cockroach detritus ($P < 0.01$), and two compounds occurring in rotting organic material, putrescine and cadaverine ($P < 0.03$). These results suggest that *P.*

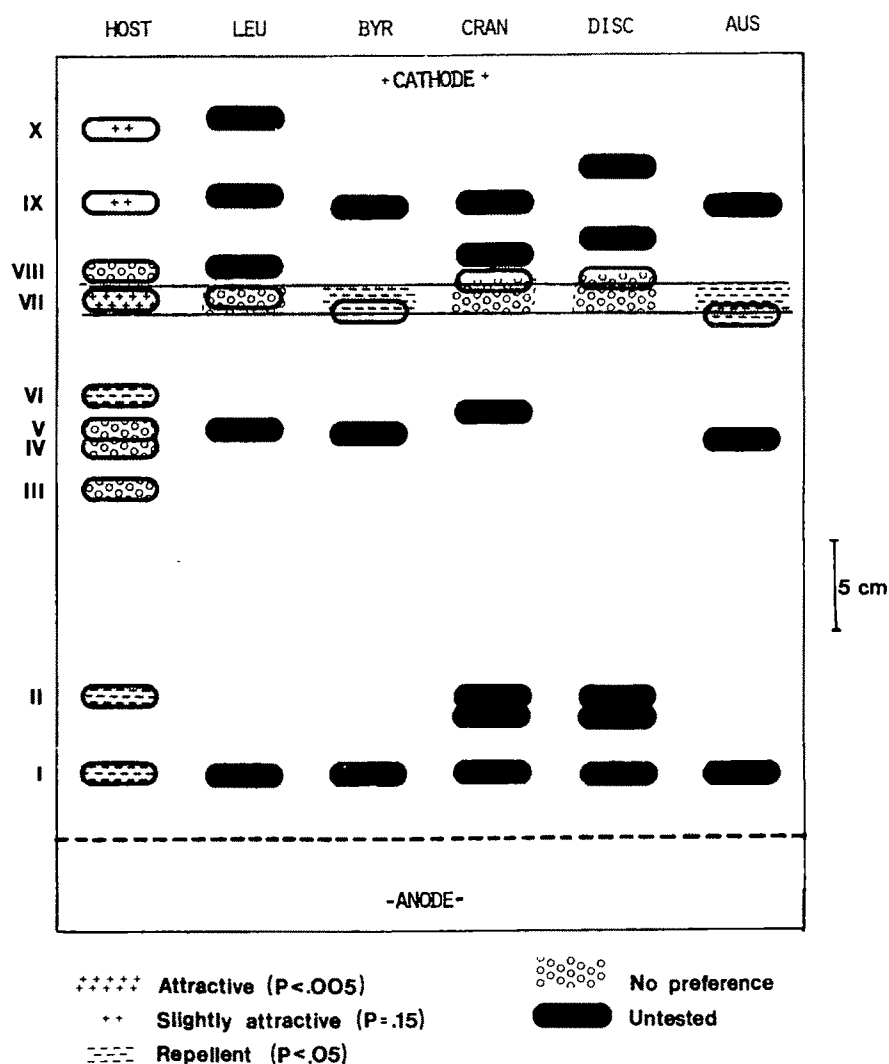


Fig. 1 Separation of nauphoetamine, a host-specific cue. Map of the components from cockroach extracts indicated by ninhydrin after high voltage paper electrophoresis (2–1/2 kV, 30 min, pH 6). Extracts were applied in equivalent concentrations on the dotted line after treatment with gel filtration. Mite responses to these components are indicated. Abbreviations: HOST, *Nauphoeta cinerea* (Olivier); LEU, *Leucophaea maderae* (Fab.); BYR, *Byrsotria fumigata* (Guérin); CRAN, *Blaberus craniifer* Burmeister; DISC, *B. discoidalis* Serville; AUS, *Periplaneta australasiae* (Fab.).

nauphoetae can recognise the proximity of potential hosts—a cockroach colony. To test this hypothesis, we isolated several hosts from their colony for 3 weeks, then tested them against colony-dwelling individuals. These mites categorically preferred the latter ($P < 0.001$); conversely, they were attracted to (but did not parasitise) a non-host cockroach (*Periplaneta australasiae* [Fab.]) that was permitted to live in a host colony for 3 weeks ($P < 0.01$).

P. nauphoetae showed a selective preference for cockroach excretants rather than faeces and detritus. They were attracted by material produced by representative species in the host's family, Blaberidae ($P < 0.05$), but not by species in other families ($P > 0.9$). In addition, host excretants were more attractive than host faeces ($P < 0.001$). These results suggest that excretants, spread over a cockroach's body by grooming, could inform parasites that a potentially suitable host was near. Supporting this hypothesis is the result that 45 of 54 mites, observed while searching for a host, made a discrete and consistent change in their searching pattern as they neared a host.

Clean filter paper placed in a cockroach colony for several weeks became impregnated with material that strongly attracted *P. nauphoetae* ($P < 0.99$). In addition, their searching behaviour was essentially the same in the presence of either this paper or a live cockroach. Extraction of this paper with acetone, methylene chloride, ether, petroleum ether, methanol and ethyl alcohol failed to remove attractive material ($P > 0.9$). Aqueous buffers at a pH greater than 7 completely removed attractant ($P < 0.005$), and boiling destroyed its activity ($P > 0.99$).

The active extract was concentrated by lyophilisation and applied to a column of Sephadex G-50 calibrated for

polysaccharides¹⁷. Column effluents were pooled into three molecular weight fractions, concentrated, and tested for their attractiveness. Only one fraction (1,500–6,500 daltons) attracted these mites ($P < 0.005$). This active fraction was lyophilised, redissolved in pyridine buffer, pH 6, and electrophoresed on paper at 2.5 kV for 30 min. Half of this electrophoretogram was stained with ninhydrin for amines, *p*-anisidine for ketoses¹⁸, or Morgan–Elson reagents for hexosamines¹⁹. Ninhydrin indicated 10 cationic bands (Fig. 1). Of these, three bands—VIII, IX, and X—also reacted to the hexosamine test; there was no reaction to the ketose test. The other half of the electrophoretogram was cut into 0.25-cm² pieces and assayed in preference tests. Only band VII strongly attracted *P. nauphoetae* ($P < 0.005$); two other bands (IX and X) may have been weakly attractive ($P = 0.15$). We have observed the band patterns in Fig. 1 in 23 runs using five different extracts.

To determine whether the attractant isolated by electrophoresis is unique to the host, we collected material from five non-host species representing the major phylogenetic lines of cockroaches. These materials were extracted, gel filtered and electrophoresed exactly as was the host material. Electrophoretograms were marked with host extract, stained with ninhydrin, and material from non-host species occurring around band VII was offered to mites (Fig. 1). None of this material was attractive ($P > 0.9$), including one band with the same mobility as the marker; material from two species was slightly repellent. From these experiments we conclude that band VII from *N. cinerea* contains a host-specific attractant.

Further chemical analysis of band VII is planned. Preliminary results by ion exchange chromatography, thin-

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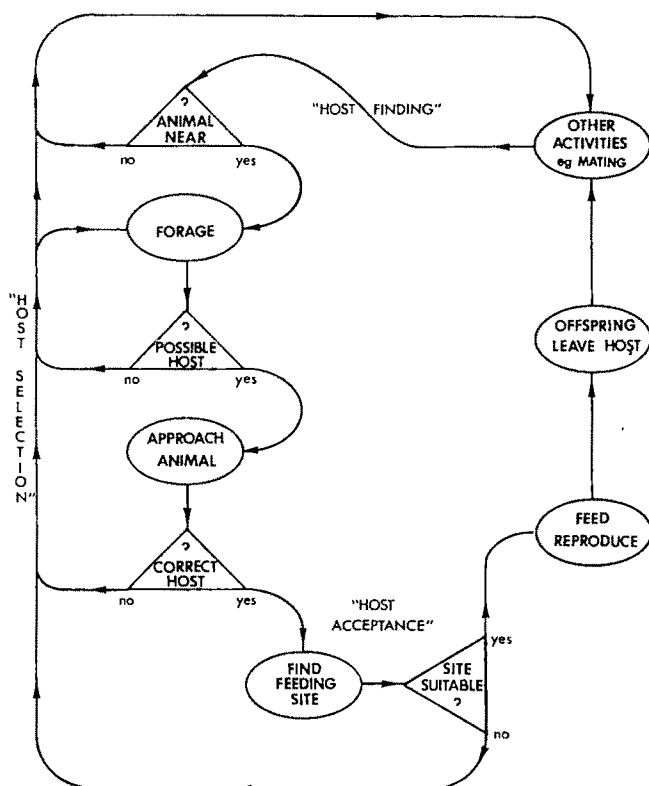


Fig. 2 A flow chart showing host discrimination. Host discrimination by *P. nauphoetae* is viewed as three consecutive processes indicated by "...". Ovals indicate simple activities while triangles represent behaviour requiring chemosensory discrimination

layer chromatography, spectroscopy and spot testing suggest that it is a glycosaminoglycan, a poly-amino sugar. The only definite conclusion is, however, that band VII is a polar macromolecule which contains a primary or secondary amine and has a molecular weight between 1,500 and 6,500. Considering these facts, and the source of the material, we propose the name nauphoetamine.

Three chemically mediated levels of discrimination were used by these parasites. The probable relationship between mite behaviour and the process of host discrimination is shown diagrammatically in Fig. 2. The first level is host proximity for which faecal odour and colony detritus seem to be cues. Perception of these cues initiates a random, undirected search (foraging). In time, this activity brings it close enough to a cockroach (or other animal), to determine if it is a potential host—the second level of discrimination. Appropriate chemical cues, for example, cockroach expectorants, result in a direct orientation. In the final approach the third level of discrimination occurs: the parasite can determine through perception of nauphoetamine whether the cockroach is the correct species. If not, it resumes foraging or engages in some other activity. If so, it moves to the abdomen, grooms its legs, and attempts to find an unoccupied feeding site^{7,8}. If unsuccessful, the mite abandons the cockroach and continues to forage. If a feeding site is found, however, the process of host discrimination is consummated by attachment which may be followed by feeding and eventual reproduction.

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Cell within a cell or a circulating cell pair

I WISH to report that one of the coelomocytes of *Phascolosoma agassizii* Keferstein, 1866¹ (collected from Arroyo de Frioles State Beach, California) which was previously considered to be one granulocyte is seen, under the electron microscope, to consist of two separate cells in a unique relationship.

The most unusual and distinctive feature of this cell pair is that there are two structurally separate cells, one within the other. In more than 100 electron micrographs of such cell pairs chosen at random, and in five pairs serially sectioned, no cell junctions, cytoplasmic bridges or points of fusion between the two cells were found.

The external cell (E) is a complete cell containing a nucleus (NE) and a scant rim of cytoplasm which surrounds the internal cell (Fig. 1). The cytoplasm of the external cell (Fig. 2) contains a Golgi complex (Gc), mitochondria, smooth and rough endoplasmic reticulum, free ribosomes and small electron-dense granules, as well as larger granules which are similar in appearance to vertebrate lipofuscin granules (Lf). At high magnification, small fenestrations 0.018 μ m wide are occasionally distinguishable along the external plasma membrane (arrows). This may be an artefact resulting from the plane of sectioning of the irregular buffy-coated outer membrane.

The external cell is separated from the complete internal cell by an extracellular space averaging 0.07 μ m wide filled with a fibrillar reticulum (Fig. 2).

The cytoplasm of the enclosed cell is almost filled with large granules of various densities. The outer granule membranes are often lined with ribosomes (not visible in the fields shown). The nucleus of this cell (NI) is displaced to a site near the border of the external cell. The organelles—a Golgi complex, mitochondria (M), rough endoplasmic reticulum, free ribosomes, microtubules and glycogen (Figs 1 and 2)—are concentrated around the nucleus where new granules can often be seen forming from the Golgi complex.

I observed no granules being extruded from the cells or disruption of the granules during clotting of coelomic fluid. But during routine processing for electron microscopy, the

granules of the enclosed cell often disrupted, leaving the associated cell, reticulum fibres and enclosed cytoplasmic membrane. No cells were observed in mitosis and the GAC (granulocyte with its associated cell) was non-phagocytic.

Of the four morphologically distinguishable cell types found in the coelomic fluid of *P. agassizii* (omitting gametes) the cell within a cell was the least common. For every three ciliated urns, 30 granulocytes and 566 haemocytes, there was only one GAC (counts taken from 25 pooled blood samples, four worms each). The GAC ranged in size from $26\mu\text{m} \times 15\mu\text{m}$ to twice this length and width. In preparations of whole cells stained with Wright's blood stain two nuclei were seen per GAC. The nuclei were observed in all possible positions relative to each other, from close together to opposite poles (Figs 1 (inset) and 2).

In paraffin sections, 4–6 μm , the granules in the enclosed cell exhibited two staining reactions with periodic acid-Schiff (PAS), Giemsa and trichrome stains². They were either PAS positive or negative; PAS-negative granules stained orange with PAS-orange G. With Giemsa the granules stained acidophilic or basophilic, and with trichrome, they stained red or green. One cell sometimes exhibited granules with both staining properties although homogeneously stained granules were the most common form. No histochemical stain differentiated the internal granulocyte from its external associated cell.

When 0.5- μm sections embedded in Araldite and stained with Toluidine blue were viewed by light microscopy, the external associated cell was not visible unless some internal granules had been disrupted. In most GACs, the granules of the internal cell obscured all other cytoplasmic features (inset, Fig. 1).

In unfixed preparations of whole cells, the GAC was reactive for both lipase³ and alkaline phosphatase⁴. Light microscopy indicated that all large internal granules were positive for lipase whereas the alkaline phosphatase reaction was random, often appearing more prominent around the periphery.

A circulating cell pair is an unusual finding. There is no report in the literature on invertebrates. Emperipolesis—cells within cells—has been reported in tumour cells⁵ of vertebrates and as a transitory state for migrating lymphocytes passing through the endothelial cells of post-capillary venules in lymph nodes⁶.

A diverse population of granulocytes has been observed

Fig. 1 Electron micrograph of a typical GAC. The cytoplasm of the inner cell, the granulocyte, is packed with dense granules. The associated cell is composed of a nucleus with scant cytoplasm completely surrounding the granulocyte. E, external cell; NE, nucleus of external cell; I, internal cell; Gc, Golgi complex; NI, nucleus of internal cell. —, Fenestration of external plasma membrane. Inset, toluidine-blue-stained Araldite section of a typical GAC.

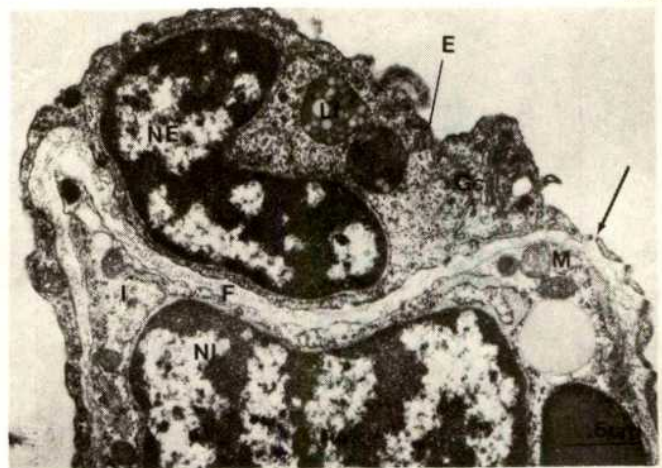
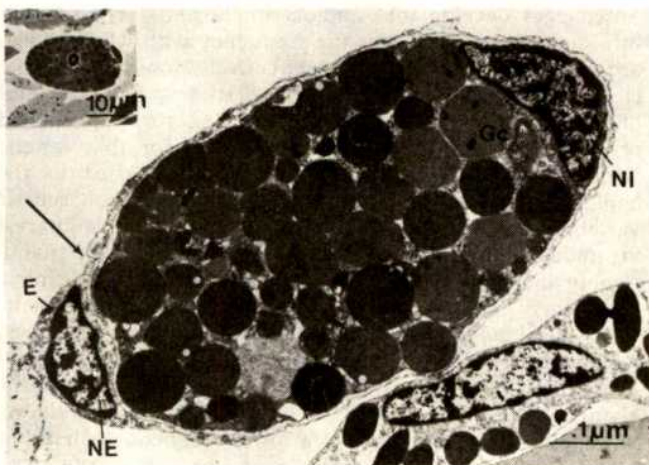


Fig. 2 Electron micrograph of the nuclear area of the GAC. Reticular fibrils can be seen separating the two cells. Lf, lipofusein granules; M, mitochondrion; F, fibrillar reticulum. Other labels as for Fig. 1.

in various sipunculid species^{7–11}. One group of granulocytes is described as multinucleate, larger with larger granules and differing in its acidophilic staining and non-phagocytic nature; but using electron microscopy it has been shown that in *P. agassizii* these are actually two associated cells. The different histochemical properties of this cell type and occasionally within a single cell with either the PAS, trichrome or Giemsa stain, could indicate different stages in packaging and condensation of a mucoprotein granule product. Variations in density revealed by electron microscopy, and the formation of new granules from the Golgi complex show a developmental sequence in the granule population. The purpose of the granules of the internal cell and the special conditions under which they might be released is not known. The granulocyte with associated cell could be a regulatory cell. The associated cell with the extracellular reticulum appears to be a physical support for the large internal granulocyte. Perhaps changes in the coelomic fluid cause changes in the external cell, resulting in lysis of the support structure and release of the internal cell's granules.

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Superior growth of the right gonad in human fetuses

RIGHT and left mammalian gonads do not usually differ noticeably either in size or development, in contrast to the situation in birds, where ovarian development is usually confined to the left side¹. Nevertheless, when the differentiation of the gonads follows an abnormal course, lateral asymmetry becomes apparent also in mammals. In human hermaphrodites, who have a testis as well as an ovary, the testis is preferentially situated on the right and the ovary on the left side^{2–4}. This

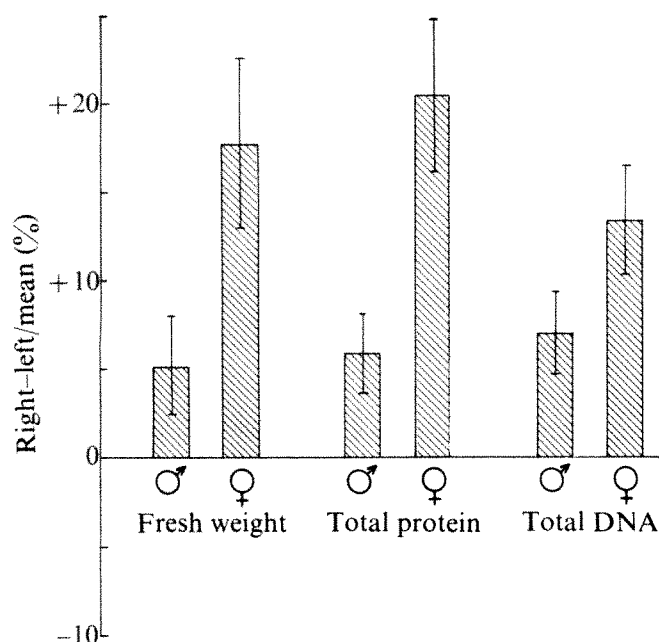


Fig. 1 Difference in fresh weight, total protein and total DNA contents between the right and left gonads of 14 male and 9 female foetuses aged between 20 and 28 weeks (estimated from crown-rump lengths). Bars represent the sums of the differences between right and left gonads, divided by the mean gonadal value, expressed in %; vertical lines represent \pm s.e. After removal, the gonads were stored in 0.03 M phosphate buffer, pH 7.0 at -15°C . Before analysis, pairs of gonads were thawed out and trimmed of extragonadal material under a dissecting microscope. Trimmed gonads were blotted dry on filter-paper, weighed and ground in the above phosphate buffer using a loose glass-glass homogeniser. Samples of the ground material were assayed in duplicate for protein (using the procedure of Lowry *et al.*¹²) and for DNA (as described by Burton¹³). Bovine albumin (Sigma) and herring sperm DNA (Sigma) Type IV were used to calibrate the protein and DNA assays respectively.

finding is independent of the chromosome constitution^{3,4}. Also, certain gonadal tumours are known to be more frequent on the right side; this applies to dysgerminomas, which are seminoma-like tumours of the ovary⁵. The question thus arises whether mammalian gonads exhibit an inherent but undetected asymmetry between right and left sides. To answer this question, we have compared right and left gonads from therapeutically aborted human foetuses, using the criteria of fresh weight, total protein and total DNA contents.

Figure 1 shows that when the values for left gonads are subtracted from the corresponding values for right gonads, the difference is positive for all three criteria in both males and females. We can thus conclude that the right gonad is superior to the left in both protein and DNA contents, and thus, one can confidently assume, in cell number. This difference seems to be greater in females than males. The larger size of the right compared with the left ovary has previously been reported in adult British horseshoe bats of the genus *Rhinopholus*, in which the follicles of the left ovary fail to mature⁶.

An increased growth rate of the right mammalian gonad, at least during part of its development, could explain the greater risk of some gonadal tumours developing on the right compared with the left side. Moreover, the preferential situation of testes on the right and ovaries on the left side in patients with hermaphroditism²⁻⁴ may be accounted for simply by the asymmetrical growth of the two gonads in normal mammalian foetuses.

Foetal testes grow markedly faster than ovaries in rats⁷ and mice⁸. We have observed a similar effect in human foetuses younger than 25 weeks. The mean gonadal weight of 10 male foetuses (average age 21.5 weeks) was 41.9 mg and that of 6 female foetuses (average age 22.0 weeks) was 25.3 mg, the difference being significant at the 1% level.

Usually the differentiation of gonads into testes and ovaries

is firmly controlled by the sex chromosome constitution, but this mechanism clearly fails to operate during the development of hermaphrodites. In this situation the differentiation of the gonads will be determined by local conditions, of which body side is one of the determining factors. Gonads situated on the right tend to become testes while those on the left are more likely to develop into ovaries. Our demonstration that the right gonad is normally the larger one seems to offer strong evidence in favour of a causal relationship between increased growth and testicular differentiation in mammals^{9,10}.

It is interesting that in chick embryos of both sexes, the left gonad is larger than the right¹¹. Since in birds the female is heterogametic with XY(ZW) sex chromosomes, in contrast to the situation in mammals, it seems that birds are a mirror image of mammals both as regards gonadal asymmetry and their sex chromosome constitution.

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Low levels of genetic heterozygosity in Hymenoptera

It has been suggested¹⁻⁴ that organisms with a haplodiploid sex determination system should have reduced genetic variability relative to diploid organisms. In haplodiploid systems such as in bees and wasps (Hymenoptera), unfertilised eggs develop into haploid males and fertilised eggs into diploid females. Thus the frequency with which alleles are exposed to selection in an effectively homozygous state is increased over comparable diploid species. A lower level of genetic variation would be expected for haplodiploid organisms, relative to diploid organisms, for that genetic variation which is not neutral and is expressed in the haploid sex. Thus, measurement of the level of genetic variability in haplodiploid species is of considerable interest in understanding the mechanisms responsible for maintaining the high levels of genetic polymorphism in diploid organisms^{5,6}. For this reason, we have measured the genetic variability in haplodiploid species using the combined techniques of electrophoresis and histochemical staining^{7,8}. As predicted by selection theory, we have shown that the Hymenoptera (haplodiploid organisms) have a lower level of genetic heterozygosity and a lower frequency of enzyme polymorphism than do diploid organisms.

Table 1 Allelic frequencies for enzyme loci resolved for seven species of solitary Hymenoptera

Enzyme* locus	<i>Stictia carolina</i> (Fabricius)	<i>Chalybion</i> <i>californicum</i> (Saussure)	Wasps <i>Sceliphron</i> <i>caementarium</i> (Dury)	<i>Scolia dubia</i> <i>dubia</i> (Say)	<i>Trypargilum</i> <i>politum</i> (Say)	Bees <i>Nomia</i> <i>heteropoda</i> (Say)	<i>Savastria</i> <i>obliqua</i> (Say)
3 HBDH	0.91 0.06 0.01 0.02	0.28 0.64 0.08	—	—	1.00	1.00	1.00
HPI	1.00	—	—	1.00	—	1.00	1.00
MDH-1	0.02 0.98	1.00	1.00	0.02 0.98	1.00	1.00	1.00
MDH-2	1.00	0.07 0.93	0.77 0.23	1.00	1.00	1.00	1.00
MDH-3	—	—	—	—	—	1.00	0.90 0.10
GAPDH-1	1.00	1.00	—	1.00	1.00	1.00	1.00
GAPDH-2	1.00	—	—	1.00	1.00	—	—
G3PDH-1	1.00	0.01 0.99	1.00	1.00	1.00	1.00	1.00
G3PDH-2	1.00	1.00	1.00	1.00	0.47 0.53	1.00	1.00
G3PDH-3	—	—	—	1.00	1.00	—	1.00
LDH	0.91 0.09	1.00	1.00	—	0.93 0.07	1.00	0.03 0.97
GDH	1.00	1.00	1.00	—	1.00	1.00	1.00
G6PDH	—	—	—	—	0.04 0.96	0.13 0.17 0.70	0.07 0.06 0.03 0.84
PGM-1	0.02 0.98	—	1.00	1.00	0.91 0.09	—	0.97 0.03
PGM-2	1.00	—	1.00	1.00	1.00	—	1.00
6 PGDH	0.40 0.60	0.98 0.02	—	0.10 0.90	0.07 0.91 0.02	—	—
IDH-1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IDH-2	1.00	1.00	1.00	—	1.00	—	—
FO	1.00	1.00	—	—	—	—	—
EST-1	0.03 0.97	1.00	1.00	0.65 0.35	1.00	0.55 0.32 0.13	1.00
EST-2	—	0.86 0.08 0.06	0.52 0.36 0.12	0.04 0.96	0.04 0.96	1.00	—
EST-3	—	0.14 0.86	—	1.00	1.00	1.00	—
EST-4	—	1.00	—	—	—	—	—
Alleles sampled	120	103	107	92	68	60	60
Number of loci	17	16	12	15	19	15	16
Frequency of polymorphic loci	0.35	0.37	0.17	0.27	0.32	0.13	0.25
Mean heterozygosity	0.056	0.073	0.078	0.051	0.059	0.070	0.038
S.e.m. heterozygosity	0.029	0.036	0.055	0.032	0.028	0.048	0.021

Allelic frequencies for enzyme loci resolved for seven species of solitary Hymenoptera. Although many precedents indicate that specific enzyme phenotypes represent at least one locus, in the absence of genetic crosses or allelic variation, it is difficult to determine whether each isozyme observed is coded in a separate locus. We have therefore been conservative in our estimates. The numerical designation assigned to multiple alleles and multiple loci coding the same enzyme function are based on their relative electrophoretic mobility with '1' being the most anodal. Enzyme (and coenzyme) used: 3 HBDH, 3-hydroxybutyrate dehydrogenase (NAD); HPI, hexosephosphate isomerase; MDH, malate dehydrogenase (NAD); GAPDH, glyceraldehyde-phosphate dehydrogenase (NADP); G3PDH, glycerol-3-phosphate dehydrogenase (NAD); LDH, lactate dehydrogenase (NAD); GDH, glutamate dehydrogenase (NAD); G6PDH, glucose-6-phosphate dehydrogenase (NADP); PGM, phosphoglucomutase; 6 PGDH, 6-phosphogluconate dehydrogenase (NADP); IDH, isocitrate dehydrogenase (NADP); FO, formazan oxidase (tetrazolium oxidase); EST, esterase (nonspecific esterases).

The results of this study for seven species of Hymenoptera are shown in Table 1. Each population represented was collected during a single day at one locality. The mean frequency of polymorphic loci and the mean heterozygosity per locus for the haplodiploid species is significantly lower than those for diploid organisms (Table 2). These results are consistent with substantial portions of observed enzyme variation being selectively not neutral. In addition to detecting selection against specific allelic isozymes, we may also be detecting the results of selection against other genes which are closely linked to those coding the allelic isozymes investigated. Other factors, such as small population size and inbreeding, could depress levels of polymorphism. This explanation is made less likely, however, by the fact that

the Hymenoptera species investigated are solitary and powerful flyers found over wide geographical areas. Partial replicates of the data in Table 1 were done on separate populations of *Sceliphron caementarium*, *Scolia dubia* and *Trypargilum politum*. No significant inbreeding or inter-population differences were found.

It is interesting that the variation in mean heterozygosity for the seven Hymenoptera species representing two superfamilies and seven genera is less than that for seven species within the single genus *Drosophila* and, in addition, is less than that for four species from the rodent genera *Mus* and *Peromyscus* (Table 2). It has been argued that the more homogeneous an environment, the smaller the store of genetic variation maintained⁹⁻¹¹. For our data to be

Table 2 Values for the average frequency of polymorphic loci and average mean heterozygosity for seven Hymenoptera species and thirteen diploid organisms

	Average frequency of polymorphic loci	Average mean heterozygosity
Hymenoptera (from Table 1)	$\bar{X} = 0.266$ s.d. = 0.090 s.e. = 0.034	0.061 0.014 0.005
Diploid organisms*	$\bar{X} = 0.392$ s.d. = 0.185 s.e. = 0.051	0.101 0.039 0.011
<i>Drosophila</i> species*	$\bar{X} = 0.507$ s.d. = 0.184 s.e. = 0.070	0.125 0.035 0.013
<i>Mus</i> and <i>Peromyscus</i> species*	$\bar{X} = 0.255$ s.d. = 0.048 s.e. = 0.024	0.079 0.027 0.013

*Data taken from R. C. Lewontin⁹.

These values were calculated separately for both seven species of *Drosophila* and four species of *Mus* and *Peromyscus*.

consistent with this theory, it would be necessary for the variation in the environmental heterogeneity to be less for the order Hymenoptera than within the genera *Mus* and *Peromyscus* or *Drosophila*.

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Inheritance of juvenile shell colour of the oyster drill *Urosalpinx cinerea*

MARINE prosobranchs have been extensively studied to elucidate the origin of their shell colour. Some authors have indicated that shell colour is in the main environmentally determined, that is, by diet^{1–5}, by light⁶, or by salinity⁷. Colour also results from direct genotypic action, for example, the change to adult coloration with maturity in some *Cypraea* species^{8–9}, or the restoration of initial colour patterns after shell repair^{10,11}.

Studies of colour-morph frequencies in natural populations of marine prosobranchs usually make the implicit assumption that colour characteristics are inherited^{12–21}, but it can be argued that the characteristic(s) used are not under close genetic control. Without information from breeding studies, the occurrence of environmental factors, which may affect the results, cannot be assessed. A more appropriate strategy would be to know the

inheritance of a characteristic before attempting large scale field studies.

In the oyster drill *Urosalpinx cinerea* adult shell colour grades from brown to purple-grey, with dark purple and white shells also occurring. Juvenile shells (<15 mm shell length (SL), measured from the siphonal canal tip to the spire apex), however, appear somewhat more discontinuous in colour. Young hatching from capsules deposited in the laboratory are either brown, purple, or albino²² (M. R. Carriker, personal communication). This report describes results of laboratory breeding experiments which indicate that juvenile shell colour is inherited in a manner which can be explained using a triallelic, single-locus genetic model, with a dominance hierarchy among the alleles.

In September, 1972, young *U. cinerea* (<8 mm SL) were isolated from local populations. These animals were raised in individual isolation until the shell length was about 15 mm when they were sexed²³, marked²⁴, and combined in pairs. Pairs were reared in separate cages, and were completely immersed in heated, flowing, seawater (system of Carriker and Van Zandt²⁴) at a temperature of at least 15 °C. *Mytilus edulis*, collected from sites where *Urosalpinx* does not occur, were provided as food; thus no extraneous drills were introduced into the cages.

Egg capsules were allowed to develop in the parents' cage until tinges of pigmentation appeared in the developing shells. The capsules were removed and placed in a hatching cage (screened with stretched nylon hose; average mesh 0.5 mm). Daily sprayings prevented clogging of the screens. As young emerged from their capsules, they were removed and placed in a second, identical cage with mussels (<3 mm valve length). This precluded cannibalism of newly emergent and emerging drills by already emerged siblings.

As the young grew, increasingly larger mussels were required, producing waste-removal problems in the fine-meshed cages due to low water flux. Some of the first groups of young consequently suffered extensive mortality from water fouling. Transferring the young to larger-meshed cages (1.5 mm) as soon as they were large enough to be retained (about 4 weeks after hatching) markedly decreased this mortality. Several groups of young were reared from crosses which suffered extensive mortality; usually only the first group was reared in more successful crosses.

Colour scoring of the external shell colour of newly emerged snails was carried out in seawater under a dissecting microscope, using incandescent lighting. Fourteen pairs produced an F₁ generation. Four crosses (A14, A15, A16 and A18) provided capsules in September, 1973; the remainder began ovipositing in February, 1974.

Three discrete external juvenile shell colours were observed among hatching *U. cinerea*: purple, brown and white. The data obtained were consistent with a triallelic, single-locus genetic model with complete dominance in the order purple > brown > white (Table 1).

With growth, however, subtle shell colour changes occur. For instance, purple bands normally occurring on interior surfaces of *Urosalpinx* shells, sometimes penetrate the shell and appear on the external surface, producing a brown, purple-banded snail, if the bands remain narrow. If the bands diffuse, a brown juvenile becomes a purplish adult. This external expression of normally interior bands is inherited, but more work is needed to establish the exact mode.

Additionally, a purplish hue is generally incorporated with age into the shells, eventually obscuring the juvenile colour. This may result either from genetic modifiers, or from prolonged mussel feeding, as suggested originally by Moore². The contribution of diet to adult shell colour is being investigated.

Prosobranchia in general can be split into two groups by the acid-extractability of their shell pigments. Archaeogastropod pigments which are protein-bound (chromoproteins) resist this procedure²⁶. Pigments elaborated by archaeogastropods are probably either digestive residues or end-products of aborted synthetic pathways^{26,27}. That archaeogastropod shell colour changes with diet^{1,3,5} is thus not surprising.

Table 1 F₁ colour morphs produced by the 14 *Urosalpinx cinerea* pairs

Cross	Parental/ juvenile shell colour	Colour of F ₁			Total hatched	χ^2	Probability
		Purple	Brown	White			
A3	P × B	60 (63.5)	67 (63.5)	—	127	0.38	0.9 > P > 0.5
A4	B × B	—	108 (108)	—	108	—	—
A6	P × B	23 (23)	23 (23)	—	46	0.00	0.9 > P > 0.5
A8	B × B	—	81 (81)	—	81	—	—
A9	P × P	96 (97.5)	34 (32.5)	—	130	0.09	0.9 > P > 0.5
A10	P × P	73 (70.5)	21 (23.5)	—	94	0.35	0.9 > P > 0.5
A11	B × B	—	93 (93)	—	93	—	—
A12	B × B	—	119 (119)	—	119	—	—
A13	B × P	123 (116.5)	110 (116.5)	—	223	0.72	0.5 > P > 0.1
A14	B × P	40 (39)	38 (39)	—	78	0.05	0.9 > P > 0.5
		50 (46.5)	43 (46.5)	—	93	0.52	0.5 > P > 0.1
A15	B × W	—	28 (23.5)	19 (23.5)	47	1.72	0.5 > P > 0.1
A16	P × P	70 (72.9)	27 (24.3)	—	104	0.41	0.9 > P > 0.5
A17	B × W	—	45 (49.5)	53 (49.5)	99	0.65	0.5 > P > 0.1
A18	B × B	—	95 (95)	—	95	—	—

Female genotypes are listed first in the parental/juvenile shell colour column. Only the major juvenile colour allele is given. Because of observed dominance relationships, and without F₂ colour segregation information, recessive alleles could not be definitively established in all crosses. Thus no recessive allelic assignments were made. Expected frequencies (in parentheses) were calculated assuming a single-locus, triallelic segregation model. Two groups of young were reared in cross A14 as the first batch suffered considerable mortality.

Meso- and neogastropod shell colour, however, is not totally independent^{2,4} of diet. *Polinices duplicatus* lightens the colour of its callus with a dietary shift towards increased consumption of *Mya*, but the rest of the shell remains unchanged⁴. Moore concluded that shell-banding in *Thais* results from a mussel diet². Berry and Crothers¹⁷ suggested that the capacity for band expression in *Thais* is genetically controlled, analogous to that of terrestrial pulmonates²⁸⁻³¹. Whether or not a *Thais* becomes banded after feeding on mussels thus depends on its inherited susceptibility. This illustrates the intimate relationship between genotypic and environmental components which influence prosobranch shell colour.

In *Urosalpinx*, the inherited juvenile ground colour can be modified with growth by both genetic (shell-band expression; hue modifiers) and environmental (diet) factors. The degree that each component predominates seemingly depends on the prevalence of certain dietary items. Clearly, care must be exercised in interpreting results of studies of shell colour of marine prosobranchs which assume close genetic control. Controlled breeding experiments are essential as a first step towards unravelling the complex and interrelated mechanisms that produce such morphological characters.

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Acrosomal chromocentre in newt spermiogenesis

STUDY of the distribution of satellite DNA during the later stages of spermiogenesis led to a model in which chromosomes of the mature sperm nucleus are U-shaped with posterior centromeres^{1,2}. We have now studied a closely related chromosome marker—constitutive heterochromatin—and, using C banding techniques³, have found a polarised arrangement of chromocentres in spermatids of several newt species.

Adult *Triturus alpestris*, *T. cristatus* and *T. vulgaris* were captured from their natural habitat near Ulm, south-western Germany; *T. marmoratus* was caught in southern France (Banyuls-sur-Mer) and *Diemyctilus viridescens* was obtained from a dealer. Four hours before they were killed, the animals received an intraperitoneal injection of 0.5 ml of a 3% (w/v) colchicine solution. Testes were cut into small pieces and exposed to hypotonic conditions in distilled water for 30 min. Shortly before centrifugation of the suspension, 0.1 of the volume of 1 mM CaCl₂ solution was added, to prevent clumping. Slides were fixed and prepared according to Evans *et al.*⁴. Only 15 s of denaturation in 0.07 N NaOH was needed to produce C bands by the method of Arrighi and Hsu⁵.

As in many species, including man⁶, chromosomes of *Triturus* have segments of constitutive heterochromatin chiefly in their pericentric regions⁶. Figure 1 shows heterochromatin segments on both sides of the centromeres of chromosomes in spermatogonial metaphase and of bivalents at diakinesis of spermiogenesis in *T. vulgaris*. These segments should also be stainable by C banding at later stages of spermatogenesis.

The typical arrangement of chromocentres in spermatids is shown in Fig. 2. There was conspicuous asymmetry: most of the large, irregularly shaped heterochromatin particles were in the half of the nucleus which later becomes the posterior pole. One large chromocentre,

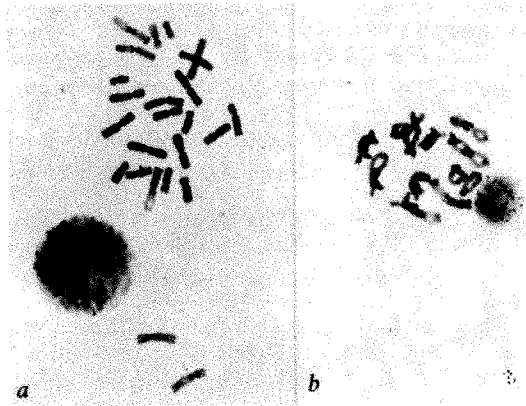


Fig. 1 Spermatogonial metaphase (a) and diakinesis (b) of *T. alpestris* showing C bands.

however, occupied the anterior pole, which later protrudes, developing a close spatial relationship to the acrosome. Of more than 500 spermatid nuclei at this stage, 67.9% had this clearly recognisable asymmetry. We have designated this isolated particulate of heterochromatin the acrosomal chromocentre. This asymmetrical distribution of the chromocentres in spermatid nuclei at this stage was evaluated quantitatively using 200 well polarised nuclei. For this purpose the nuclear area was divided into two parts, defined by a line drawn at right angles to the diameter which crosses the acrosomal chromocentre (Fig. 2a). There was an average of 11.31 large chromocentres per spermatid nucleus. This corresponds well with the haploid number of chromosomes ($n=12$) and shows that extensive fusion of chromocentres does not occur at that stage of spermiogenesis.

The average number of large chromocentres in the anterior and posterior areas of the spermatid nucleus was 1.94 and 9.37, respectively. The distribution of the dot-like small chromocentres followed a less pronounced but similar pattern.

Shortly before the stage at which the spermatid nucleus becomes asymmetrical by the formation of a protrusion at the anterior pole, the acrosomal chromocentre started to elongate and formed a rod-like structure (Fig. 2b). This extended further, concomitant with the growth of the anterior pole, and became a thread (Fig. 2c) which, because of its typical position at the end of the mature sperm nucleus, can be designated the acrosomal chromonema. Very often, a small knob remained visible in the middle of the thread, and in the best preparations a fine beaded structure could be recognised, especially along the anterior segment (Fig. 2d). A homologous structure was detected in mature sperm nuclei of *T. alpestris*, *T. cristatus*, *T. marmoratus* and *D. viridescens*. Demonstration of the acrosomal chromonema in mature sperm nuclei requires relaxation of their tightly spiralled distal part, which can be achieved by brief treatment with Ca^{2+} and by heating the slides. With the DNA-specific bis-benzimidazol stain (Hoechst 33258), the whole sperm nucleus fluoresced brightly, preventing demonstration of the acrosomal chromonema. After mild treatment with deoxyribonuclease, however, this structure became clearly visible as a brightly fluorescent thread (Fig. 3).

None of the somatic tissues examined showed the peculiar arrangement of chromocentres found in spermatids. Nuclei of cells from liver, kidney, intestine, bone marrow and oviduct have randomly distributed chromocentres after C banding. Thus, the polarised arrangement of chromocentres is specific for the postmeiotic cells during spermiogenesis.

To which chromosomes does the acrosomal chromocentre belong? As well as the segment of pericentric hetero-

chromatin, *T. vulgaris* has a heterochromatic region at the distal segment of the long arm of chromosome 1. This region was discovered by Nardi *et al.*⁶ in premeiotic metaphases and can also be recognised on the largest bivalent in Fig. 1. If the acrosomal chromocentre is formed by this block of distal heterochromatin, Macgregor and Walker's description of U-shaped chromosomes with posterior centromeres would still apply to all centromeres. If the plethodontid salamanders they investigated also have a distal block of heterochromatin forming an acrosomal chromocentre, this would probably not be detectable by their method of *in situ* hybridisation with RNA homologous to the minor component DNA. It is likely that the distal C band is built up by a different kind of satellite DNA, representing a very small part of the total DNA. An alternative model would imply that one of the centromeres migrates to the anterior pole shortly before acrosome formation. New differential staining methods (especially with fluorescent dyes) could help to identify the acrosomal chromocentre with one of the C bands in the chromosome complements of the *Triturus* species we examined. There

Fig. 2 a, Asymmetrical arrangement of chromocentres in spermatids of *T. vulgaris*. b, First stage of elongation of the acrosomal chromocentre (*T. vulgaris*). c, Growth of the anterior pole and further elongation of the acrosomal chromonema (*T. vulgaris*). d, Very late spermatid (left) and mature sperm heads of *T. marmoratus* (centre) relaxed by Ca^{2+} treatment, and of *T. vulgaris* (right) not relaxed.

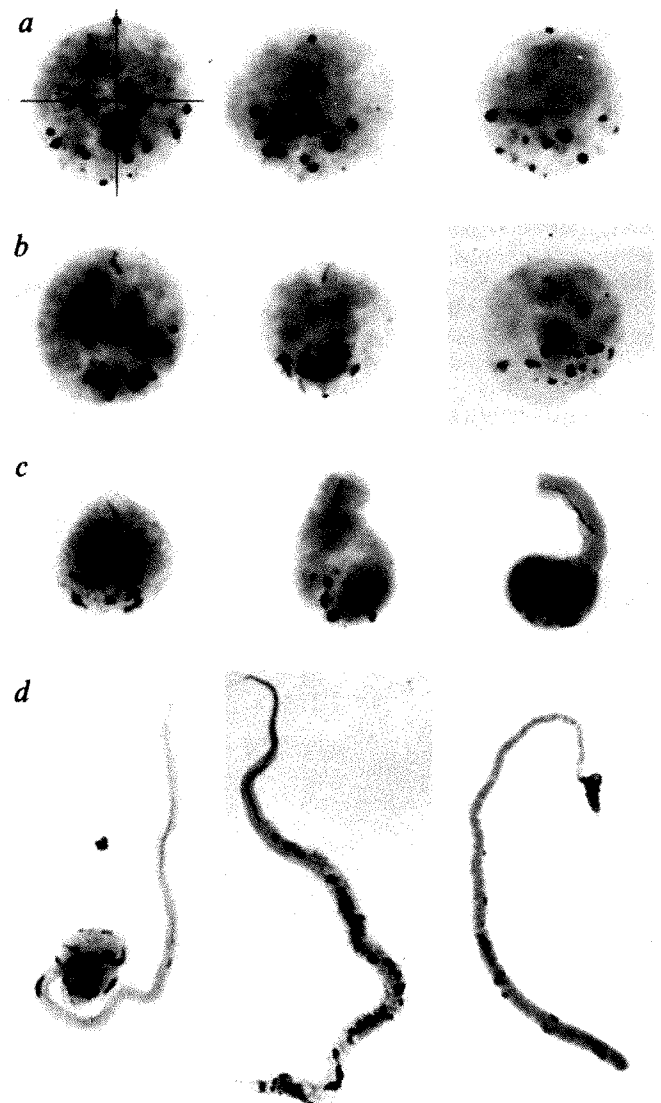




Fig. 3 Late spermatid of *D. viridescens* stained with Hoechst 33258 after mild treatment with deoxyribonuclease. The acrosomal chromonema is clearly visible as a brightly fluorescent thread in the anterior part of developing sperm head.

could be a functional correlation between the acrosomal chromocentre, its morphological alterations during the final stages of spermiogenesis and the location of the genetic information necessary for the organisation of the acrosome. This correlation is also suggested by the presence of peculiar intranuclear and extranuclear structures in differentiating spermatids of a wide variety of species. These structures are found at the part of the nuclear membrane which is first in close contact with the proacrosome granule and later with the growing acrosome⁷.

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Antibody-forming cells in human colostrum after oral immunisation

It has been suggested that feeding infants with human milk reduces the incidence of gastrointestinal infections¹. Although macrophages, lymphocytes and antibodies in the milk are probably important in this protection^{2,3}, the mechanism for the development of specific immunity to enteric pathogens is not known. Human colostrum cells synthesise

IgA (ref. 4), and we have used the haemolysis-in-gel technique to demonstrate that human colostrum contains numerous cells which produce IgA antibodies against the O antigens of commonly encountered *Escherichia coli* bacteria⁵. This suggested that the sensitised lymphocytes had either undergone antigen-induced clonal proliferation within the mammary gland or had homed to that organ from another site after becoming sensitised. Because of reports that gastrointestinal immunisation of germ-free mice led to local⁶ and systemic⁷ production of IgA antibodies, and that cells from Peyer's patches effectively repopulate the ileum of lethally-irradiated rabbits with IgA-producing cells⁸, we have examined the effect of gastrointestinal immunisation on the development of antibody-producing cells in colostrum of humans. We found that oral administration of a non-pathogenic strain of *E. coli* led to the rapid appearance of colostrum cells producing antibodies against the O antigen of the organism.

Three women from our research group consented to ingest 10^8 live *E. coli* 083 bacteria during the last month of their pregnancies. This organism had been used previously to colonise non-pregnant adults (R.M.G. *et al.*, unpublished) and many infants in the first day of life^{9,10} without harmful effects. Therefore, we felt confident that this procedure carried little risk for either the woman or the foetus. The procedure was explained to each subject and verbal consent was obtained. Immediately before ingestion, each subject took 2 g NaHCO₃ to reduce gastric acidity. Before colonisation, samples of serum, parotid fluid and colostrum were collected for the quantitation of antibodies by an enzyme-linked immunosorbent assay^{5,11}, and the *E. coli* in a stool specimen were typed with respect to the O antigen¹². In addition, colostrum was examined for lymphocytes producing antibodies to the lipopolysaccharide (LPS) from the 083 bacteria or a pool of eight more prevalent types of *E. coli* by a modification of the haemolysis-in-gel techniques^{5,13}. Each test was repeated every 3–6 d until the first week *post partum*. In two subjects, a second dose of the bacteria was administered 7 and 10 d after the first.

As previously reported⁵, when a pool of LPS derived from commonly encountered *E. coli* was used as antigen, large quantities of secretory IgA (SIgA) antibodies and numerous IgA-producing cells were found in colostrum (Fig. 1). No consistent change in these values could be related to gastrointestinal immunisation with the *E. coli* of unrelated O type.

Before ingestion of the bacteria, low levels of antibodies to the LPS of *E. coli* 083 were detected in the serum, saliva and colostrum (Fig. 1). Antibodies of the IgA class predominated in the saliva and colostrum and these seemed to be of the SIgA type since the quantitations using an anti- α chain and anti-secretory component (SC) as the enzyme carrier correlated significantly ($r = 0.561$; $P < 0.001$). *E. coli* 083 bacteria, however, were not identified in the stool and cells forming antibodies to the 083 antigen were not detected in the colostrum by the plaque assay before immunisation.

As in infants⁹ and non-pregnant adults (R.M.G. *et al.*, unpublished), ingestion of the *E. coli* 083 did not give rise to symptoms. Furthermore, colonisation with the bacteria was short lived and in none of the individuals did this bacterium dominate the aerobic faecal flora. Most frequently the 083 bacteria could only be identified by serotyping those colonies which were selected by their haemolytic capacity.

Nonetheless, beginning as early as 3 d after the ingestion of the bacteria, cells producing antibodies against the O antigen of the *E. coli* 083 bacteria were detectable in the colostrum from each of the colonised individuals. These cells formed plaques in the presence of developing antisera against α chains or SC, suggesting that the antibodies released were SIgA. The specificity of the plaques produced

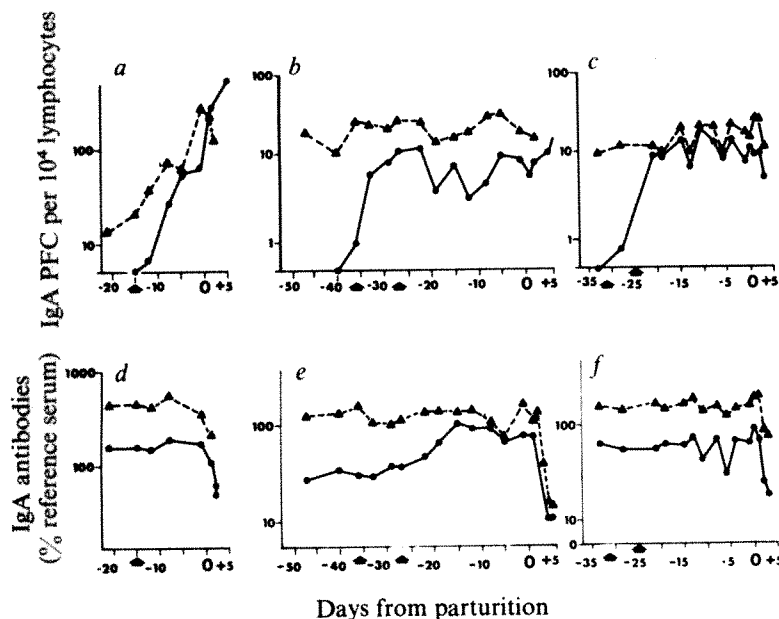


Fig. 1 a-c, Effect of gastrointestinal colonisation with *E. coli* 083 on the number of colostrual cells producing IgA antibodies against O antigen of immunising bacteria (—) or a pool of commonly encountered *E. coli* O antigens (-----). Human red blood cells (RBC) coated with O antigens or uncoated were mixed with colostrual leukocytes in an agar-containing medium. After 1 h incubation (37 °C), anti-immunoglobulin reagents, anti-IgG, anti-IgA or anti-SC (Dakopatts, Copenhagen), were added. Incubation was continued for 1 h, guinea pig complement added for 45 min and plaques counted under oblique light⁸. Significant numbers of plaques were only noted with coated RBC and anti-IgA or SC-developing sera. d-f, Quantity of colostrual IgA antibodies against the same O antigens. Alkaline phosphatase conjugated to the same anti-immunoglobulin reagent was used in the enzyme-linked immunoabsorbent assay⁹. The results are expressed as percentage of the activity of a reference serum. (a,d) (b,e) (c,f) are single individuals; arrows, the time of ingestion of bacteria; each point is the mean of duplicate determinations.

by colostrual cells was verified by inhibition with an excess of purified 083 LPS (250 $\mu\text{g ml}^{-1}$) or by inhibiting protein synthesis with puromycin (10 $\mu\text{g ml}^{-1}$). In one individual, the proportion of lymphoid cells forming IgA plaques against *E. coli* 083 increased logarithmically during the 20 d after a single antigen ingestion. At this point, the number of cells forming plaques against this antigen was greater than that against the pool of eight commonly encountered *E. coli* types (Fig. 1a). In the two individuals who received two doses of the bacteria, the peak number of cells producing antibody against the O antigen of the immunising bacteria was reached after 14 d. The number of anti-083-producing cells in their colostrum approached that of the cells producing antibody against the pooled antigens, and was maintained throughout the remainder of the observation period (Fig. 1b and c).

An increase in the level of IgA antibodies to the 083 bacteria was detected in the colostrum by the enzyme-linked immunosorbent assay after the second dose of bacteria in only one of three immunised individuals (Fig. 1b). In contrast, 083 plaques of the IgM and IgG classes never increased significantly above the background level noted in assays with uncoated erythrocytes and the levels of IgG or IgM colostrual antibodies did not change after immunisation. Further, no increase in salivary or serum antibody of any class was noted.

The disparity between the number of IgA antibody-producing cells and the antibody content of the colostrum, also noted before³, is not explained by the experiments reported here. The finding, however, provides further evidence for *de novo* synthesis of IgA antibodies by colostrual cells.

Two possible mechanisms might be considered to explain the prevalence of colostrual cells producing type-specific IgA antibodies to *E. coli* present in the gastrointestinal tract. (1) The bacteria or their LPS antigens may enter the blood from the gastrointestinal tract and cause a clonal proliferation of the antibody-producing cells or their precursors in the mammary tissue. This seems unlikely because of the mild degree of colonisation and the lack of serum antibody response to the administered organisms. (2) The immunological specificity might be transferred from the gastrointestinal tract to the breast by sensitised cells. Because of the lack of a systemic immune response to the ingested antigens, we favour this possibility. Our experiments do not indicate whether these hypothetical transferred cells are the same as those forming haemolytic plaques or whether they are cells which collaborate in the immune response.

Our observations are supported by the report of SIgA

antibodies in the milk of rabbits fed DNP-bovine γ globulin and DNP-type III pneumococcal vaccine¹⁴, and the occurrence of IgA antibodies against *Salmonella typhimurium* in milk of three women infected with this bacteria during pregnancy¹⁵. Because only free antibodies in the milk were measured in those studies, however, the possibility of transfer of antibody from the serum could not be eliminated.

Our studies indicate a close relationship between antigenic sensitisation by way of the gastrointestinal tract and the development of SIgA antibody-producing cells in the milk. We propose that this phenomenon is due to a selective transfer of sensitised lymphocytes from the gastrointestinal tract to the mammary gland. Since the mother is exposed to the microorganisms which the newborn is likely to encounter, the passive immunity provided by this mechanism could be important in the protection of the infant's gastrointestinal tract.

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Separation of antiviral activity of human interferon from cell growth inhibitory effect

It is well established that potent interferon preparations besides inducing antiviral activity¹, inhibit growth of homologous cells in culture²⁻⁴. In addition, interferon increases the susceptibility of cells to the toxic effects of synthetic double-stranded RNA⁵ and to sensitised lymphocytes⁶, enhances phagocytic activity⁷ and stimulates interferon production by priming⁸. In most studies a close correlation was found between antiviral and non-antiviral activities. The correlation is independent of the degree of purification, and efforts at separation have largely been unsuccessful^{2,5,8,9}, indicating that the effects are attributable to the same substance.

Contradictory findings have also been reported. Some interferon preparations were apparently enriched in one of the effects¹⁰, others contained factors antagonistic to the non-antiviral activities³. A partial separation of effects has also been described¹¹. All these experiments were done with the mouse interferon system. The few data obtained on human interferon^{4,12-14}, although comparable with those found with mouse interferon, are insufficient to characterise the system. Although physicochemical differences have been described, in principle interferons from different species are believed to have an identical mode of action. Recently initiated clinical trials have accentuated the need for a closer examination of the human system. The results reported in this study indicate that it is possible to separate the growth inhibitory effect of human interferon from the antiviral activity by a method based on the selective adsorption of human interferon to albumin. Human interferon was obtained from the supernatant of Sendai virus-induced leukocytes¹⁵. Antiviral activity was assayed by the infectivity inhibition microtest¹⁶. Titres were adjusted to the international standard preparation, 69/19. The preparations were simultaneously assayed for their effect on cell growth. Aliquots of 10⁵ human embryo lung cells (HEL) were seeded into at least five tubes per sample with medium containing interferon, and

Table 1 Growth inhibitory effect promoted by 50 U interferon per ml from different steps during purification of human leukocyte interferon

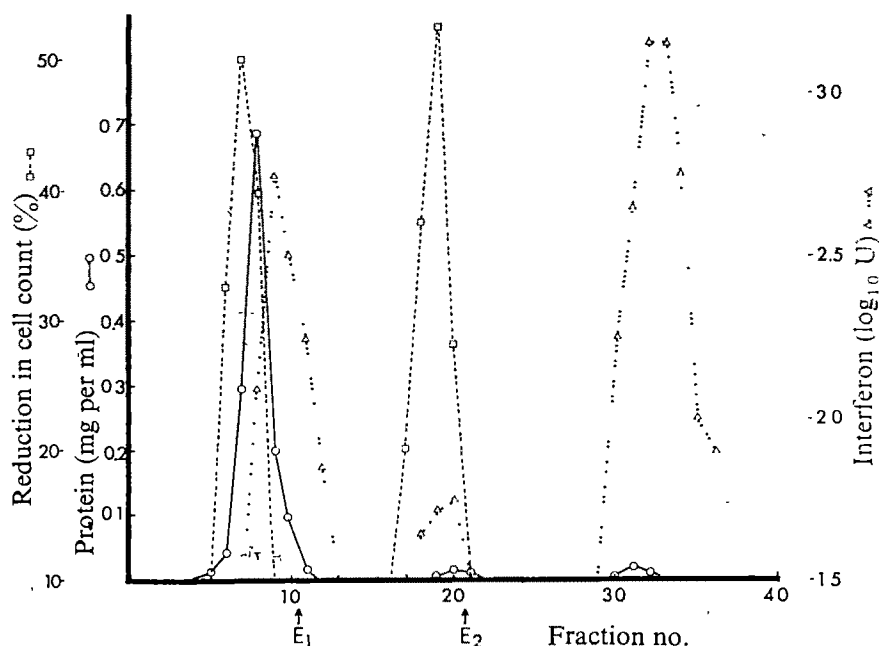
Purification step	Interferon (U per mg protein)	Reduction in cell count (%)
Crude interferon	0.6 × 10 ³	24
Alcoholic precipitate at pH 4.5	2.0 × 10 ³	22.5
Alcoholic precipitate at pH 5.7	3.0 × 10 ³	23
Alcoholic precipitate at pH 8.1	6.0 × 10 ³	24
Partially purified (Cantell)	7.0 × 10 ³	28

Interferon was induced in human leukocytes by Sendai virus¹⁵. Crude interferon preparations were precipitated with 0.5 M KSCN, the precipitate dissolved in acid alcohol and purified by stepwise elevation of the pH. The partially purified (Cantell) preparation was produced in the same way by Dr K. Cantell, University of Helsinki, Finland. 10⁵ HEL cells per tube were seeded in Eagle's MEM with 2% calf serum. After 3 d of incubation at 37 °C the tubes were treated with trypsin-versene and counted in a haemocytometer. Control tubes without interferon represent 100%.

into control tubes without interferon. Cultures were incubated at 37 °C in a 5% CO₂ atmosphere. On the basis of preliminary studies indicating a straight line dose dependence, 50 U interferon per ml were routinely used. For growth curves at least five tubes were counted every day after trypsin-versene treatment. In a typical experiment 78 × 10³, 130 × 10³, and 244 × 10³ cells per tube were found for the controls at day 1, 2, and 3 respectively against 88 × 10³, 111 × 10³, and 162 × 10³ respectively for the interferon-treated cells. The standard deviation never exceeded 10%. By further incubation the difference between interferon-treated cells and controls levelled off as the control cells entered the stationary phase. On the basis of these results 3 d of incubation were routinely used in tests for cell growth inhibition.

The consecutive steps of purification of the crude interferon preparations, including a highly purified sample from Dr K. Cantell¹⁵ were tested for antiviral and cell growth inhibitory activities (Table 1). The effect on cell growth was strictly correlated to the antiviral effect, regardless of the degree of purification, in accordance with earlier findings^{2,3,5,8}. Interferon preparations were applied to an albumin-Agarose column¹⁷ (Fig. 1). At low ionic strength (phosphate-buffered saline (PBS) (0.15 M NaCl) pH 7.4) the bulk of contaminating proteins could be washed off the column together with unattached interferon. The major part of interferon activity was bound to the column. The eluant was then changed to PBS (1 M NaCl)

Fig. 1 Affinity chromatography of human leukocyte interferon on albumin-coupled Agarose column. CNBr activated Sepharose 4B was swollen in dilute HCl and coupled with bovine albumin by mixing for 2 h. 9 mg of albumin were bound to 1 ml of Agarose beads. Crude interferon, 2 × 10⁴ U in 2 ml, was applied to a PBS equilibrated column. The column was first washed with phosphate-buffered 0.15 M NaCl at pH 7.4. The eluant was changed after the tenth fraction (5 ml each) to PBS (1 M NaCl) at pH 7.4, and after the twentieth fraction to 33% ethylene glycol in PBS (1 M NaCl). All fractions were tested for protein content by the method of Lowry²⁰, antiviral activity, and effect on cell multiplication.



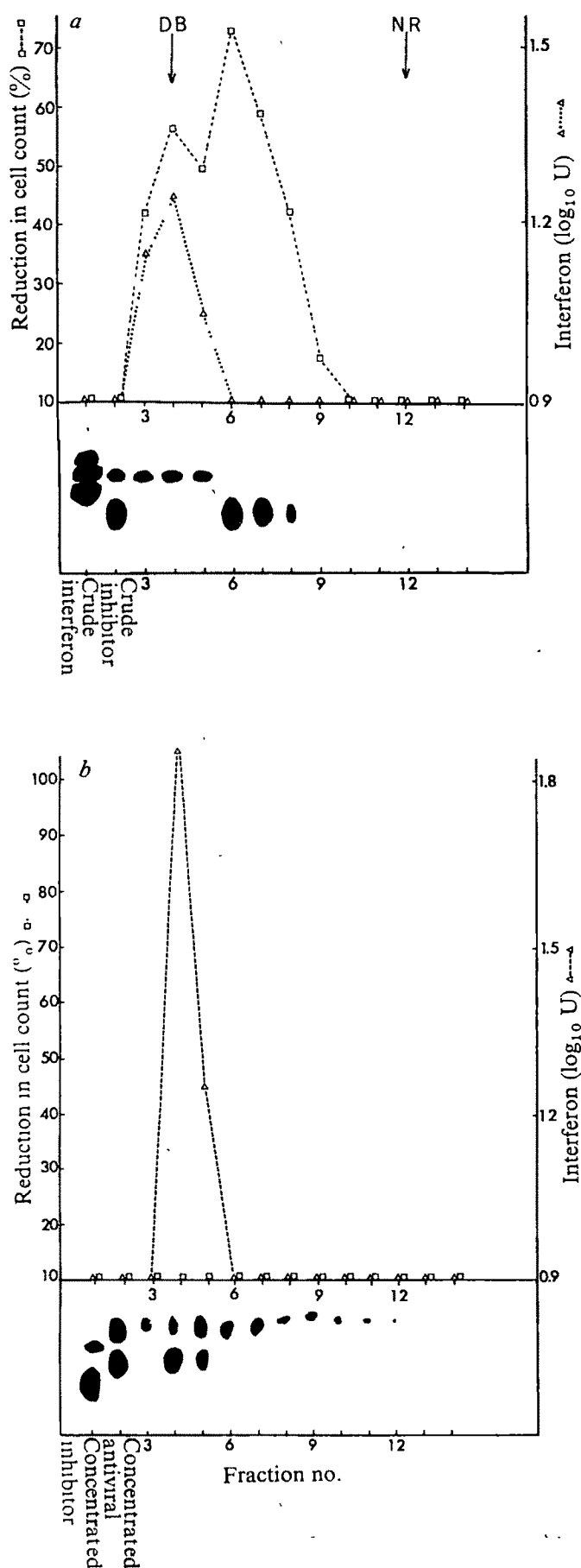


Fig. 2 *a*, Separation of antiviral activity from the effect on cell growth on a Sephadex G-25 column. The fractions from the affinity chromatography, containing cell growth inhibitory effect (Fig. 1, fractions 17-20), were pooled, concentrated by lyophilisation and applied to a Sephadex G-25 column. Each fraction (5 ml) was tested for antiviral activity and for effect on growth of HEL cells in 1:20 dilution. The arrows indicate the appearance of the included markers: DB, Dextran blue, molecular weight 150,000; NR, neutral red, molecular weight 1,000. Thin-layer chromatography of the fractions on Eastman cellulose sheets, developed in alcohol-acetic acid-4% NaOH (50:20:30) and detected in ninhydrin reagents, is shown in the lower part of the figure. Crude interferon and concentrated pool of the fractions containing cell growth inhibitory effect are included as controls. *b*, Analysis of the fractions from the affinity chromatography, containing antiviral activity (Fig. 1, fractions 30-34) on a Sephadex G-25 column. Thin-layer chromatography of the fractions is shown on the lower part of the figure. Preparation and analysis of the fractions as for *a*.

at pH 7.4, which eluted a sharp peak of cell growth inhibitory activity with only a small antiviral effect. The remaining antiviral activity was eluted in a single peak with 33% ethylene glycol in phosphate-buffered 1 M NaCl (v/v) at pH 7.4. These fractions had no effect on cell growth in dilutions containing 100 U interferon, expected to depress the cell growth by 30-40%. Nor was there any detectable effect on the growth of HeLa cells, which were more sensitive than HEL to the effect of unseparated preparations. Control preparations obtained from the supernatant of uninduced leukocytes and chromatographed as the interferon preparations, showed neither antiviral activity against VSV nor effect on the growth of HEL or HeLa cells.

The fractions showing growth inhibitory effect were examined further. Dialysis against PBS eliminated the growth inhibitory effect, suggesting a low molecular substance, distinct from the interferon molecule with reported molecular weight ~ 20,000 (refs 18 and 19). Pooled fractions, concentrated by lyophilisation, were applied to a Sephadex G-25 column (Fig. 2*a*). Antiviral and some growth inhibitory effect were eluted by PBS in the void volume and resulted in a single spot in the thin-layer chromatogram (Fig. 2*a*, lower part). Growth inhibitory effect without detectable antiviral activity was eluted at molecular weight ~ 3,200, and these fractions gave rise to single spots in the chromatogram, but with a different retention factor, indicating the separation of the two effects in distinct molecular components. The antiviral fractions obtained by the affinity chromatography were treated by the same process (Fig. 2*b*). The antiviral activity was eluted in the void volume, and no cell growth inhibition could be detected in any of the fractions. The results were confirmed by thin-layer chromatography: the antiviral fractions resulted in spots with retention factors distinct from those fractions where both activities were detected.

Dialysis of crude human leukocyte interferon against 1 M NaCl resulted in the reduction of the growth inhibition to less than half the normal activity. When the interferon was unfolded by treatment with 5 M urea before dialysis against 1 M NaCl, however, the growth inhibitory activity was completely lost. This result may suggest that the growth inhibitory component is situated inside the interferon molecule and may be released only by forces, which alter the tertiary structure.

The results reported here indicate that the antiviral activity of interferon can be separated from the effect on cell growth by a relatively simple procedure. The separation is probably caused by the high ionic strength indicating that the molecules responsible for the two different effects are bound to each other by electrostatic bonds.

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Specific afferent interference by antiserum of *in vivo* immunity

ANTISERUM can potentially interfere with effective immunity at three different parts of the immune response: afferent, central and efferent¹⁻⁶. Coating or masking of antigenic sites on tumour cells with antiserum could render the cells non-immunogenic^{1,2}—called afferent block—or protect them from attack by immune lymphocytes^{3,4}—called efferent block. In central interference, the ability of the lymphocytes to become sensitised against tumour antigens is suppressed, or immune cells are made non-reactive by the humoral factors^{5,6}. Most early studies in this area were performed with tumour allografts or used antisera with antigenic specificities which were not well characterised. Only a few studies have been performed with syngeneic tumours^{7,8}. Because of the complexity of surface antigens expressed on tumour cells, the effects of the antisera on the tumour-associated transplantation antigen (TATA) was therefore difficult to assess.

We have studied the biological effect of well characterised antisera on the induction of *in vivo* immunity to syngeneic tumours. Three such sera were used. (1) Anti-Friend virus-induced leukaemia serum (AFLS), produced by syngeneic immunisation of C57BL/6 mice with FBL-5 cells, a Friend virus-induced leukaemia. This serum recognised three antigenic specificities: Friend type specific, FMR (Friend, Moloney, Rauscher) and cross-reactive foetal antigens⁹. (2) Anti-SV40 tumour serum (ASVS) produced by immunisation of (BALB/c × C57BL/6)F₁ with a syngeneic SV40 tumour. This serum would react chiefly with a SV40 tumour-associated cell surface antigen (TASA)¹⁰. (3) Anti-foetal serum (AFS), produced by immunisation of C57BL/6 mice with syngeneic foetuses. This serum only recognised the foetal antigens expressed on foetal cells or various tumour cells¹¹.

The FBL-3 cells have been shown to be very tumorigenic (intraperitoneal inoculation of 1×10^3 cells would produce progressive tumour growth), and it was also very immunogenic and immunosensitive (immunisation with 1×10^4 X-irradiated cells could produce protection against intraperitoneal inoculation of 1×10^6 FBL-3 cells)¹², and therefore, the FBL-3 cells were very suitable for tests of *in vivo* tumour immunity. In our study, sera of various sources were preincubated with 10,000 r. X-irradiated FBL-3 cells. Serum-treated and untreated cells were then inoculated into B6 mice to determine the effect of serum treatment on the ability of these cells to produce *in vivo* tumour immunity. The results are summarised in Table 1. In experiment 1, 1×10^4 or more untreated irradiated FBL-3 were needed to produce significant protection against challenge with FBL-3 cells. In contrast, 1×10^4 serum-treated cells produced considerably less protection. This blocking of the immuno-

Table 1 Blocking of *in vivo* tumour immunity against FBL-3 cells

Treatment*	Immunisation No. of cells†	Tumour take after challenge (%)‡	%Protection	%Blocking†§
Experiment 1				
—	1×10^3	13/15 (87)	13	—
AFLS	1×10^3	11/15 (73)	27	—108
—	1×10^4	5/30 (17)	83	—
AFLS	1×10^4	25/34 (74) ¶	26 ¶	69 ¶
—	1×10^5	1/23 (04)	96	—
AFLS	1×10^5	8/35 (23)	77	20
—	—	10/10 (100)	—	—
Experiment 2				
—	5×10^4	1/17 (06)	94	—
NMS	5×10^4	2/20 (10)	90	4
AFLS	5×10^4	8/15 (53) ¶	47 ¶	50 ¶
—	—	10/10 (100)	—	—
Experiment 3				
NMS	3×10^4	4/16 (25)	75	—
ASVS	3×10^4	6/17 (35)	65	13
AFS	3×10^4	8/19 (42)	58	23
AFLS	3×10^4	14/19 (74) ¶	26 ¶	65 ¶
—	—	19/19 (100)	—	—

Experiments 1, 2 and 3 were separate, individual experiments. NMS, normal mouse serum. In experiments 2 and 3, 0.4 ml undiluted serum was incubated with 1×10^6 10,000 r. X-irradiated FBL-3 cells at 37 °C for 60 min.

* In experiment 1, 0.2 ml undiluted serum was incubated with 5×10^4 , 5×10^5 or 5×10^6 10,000 r. X-irradiated FBL-3 cells, respectively, at 37 °C for 60 min. After treatment, they were resuspended to 10 ml in Hanks' balanced salt solution, and 0.2 ml of each preparation was inoculated intraperitoneally into each mouse (the mice received 1×10^3 , 1×10^4 and 1×10^5 cells, respectively). Untreated X-irradiated FBL-3 cells were given in the same manner.

† Inoculated intraperitoneally with indicated number of 10,000 r. X-irradiated cells.

‡ Mice were challenged intraperitoneally with viable FBL-3 cells. The challenge doses were 1×10^4 – 1×10^6 in experiment 1, 3×10^5 – 3×10^6 in experiment 2, and 3×10^6 cells in experiment 3. The denominator indicates total number of mice challenged, the numerator indicates the number of mice with tumour growth. The figures in parentheses indicate the percentage of mice with tumours. Mice were observed for tumour incidence for 30–60 d. All control mice died within 14–20 d of challenge.

§ (% Protection obtained with) – (% Protection obtained with) untreated cells treated cells

% Protection obtained with untreated cells

¶ $P \leq 0.01$ by χ^2 test, by comparing the group inoculated with antiserum treated cells with the group inoculated with untreated cells (experiments 1 and 2), or with the group inoculated with normal serum-treated cells (experiment 3). In experiment 1, comparisons were made between groups receiving the same number of cells.

genicity by the antiserum was overcome by immunising with 1×10^5 treated cells. In experiment 2, blocking of immunogenicity was again shown to occur with 5×10^4 cells treated with AFLS, but not with cells treated with normal serum. The specificity of the blocking of anti-tumour protection was further shown in experiment 3. Only AFLS produced a significant interference with the immunisation. Sera produced by immunisation with SV40 (ASVS) or by foetal tissue (AFS) had no significant effect.

To determine whether the blocking effect by AFLS could be explained by toxicity to FBL-3 cells, the following experiments were performed. First, FBL-3 cells treated with AFLS were shown to retain viability *in vitro*, as measured by Trypan blue dye exclusion. Second, normal or immune C57BL/6 mice were challenged intraperitoneally with untreated or serum-treated viable FBL-3. The results are summarised in Table 2. Serum treatment had no effect on unimmunised mice, which indicated that AFLS did not cause significant killing of the FBL-3 cells *in vivo*. Such serum treatment, however, increased the protection in immune mice. The mechanism of this potentiation of established *in vivo* immunity was not clear. It may be due to some augmented effect exerted by antiserum on the effector cells; or the presence of unblocking antibodies in

Table 2 Direct effect of antiserum on growth of FBL-3 cells in normal and immune hosts

Immunisation*	Treatment of challenge cells†	Tumour take‡
—	—	14/14 (100)
—	AFLS	14/14 (100)
FBL-3	—	8/18 (44)§
FBL-3	NMS	8/17 (47)§
FBL-3	AFLS	2/18 (11)§ ¶

* Inoculated intraperitoneally with 5×10^4 , 10,000 r X-irradiated FBL-3 cells

† 1×10^6 viable FBL-3 cells were incubated with 0.4 ml of undiluted serum at 37 °C for 60 min.

‡ Same as Table 1.

§ $P < 0.05$ by χ^2 test, by comparing with unimmunised mice challenged with untreated FBL-3 cells.

¶ $P < 0.05$ by χ^2 test, by comparing with immunised mice challenged with untreated FBL-3 cells.

this serum to offset the blocking antibody which might be present in these immune hosts^{13,14}. It seemed to be different from the antibody-dependent, cell-mediated cytotoxicity^{15,16}, however, in which specific cytotoxicity could be produced by the interaction of non-sensitised lymphoid cells and specific antiserum.

Our results indicated that specific antiserum could produce afferent interference with *in vivo* tumour immunity by masking the specific antigenic sites and thereby block the immunogenicity of the tumour cells. This effect was not likely to be due to central interference, since inoculation of 1×10^5 serum-treated cells, which involves administration of at least the same amount of antiserum on the coated tumour cells, had no significant blocking effect (Table 1). These results are more consistent with insufficient masking of the larger number of tumour cells by the amount of available antibodies. This finding also indicated that the blocking was not due to an effect on the effector cells; if it was due to elution of antiserum from the coated tumour cells to act on the effector cells, then blocking should be obtained with 1×10^5 treated cells. The mechanism of blocking also did not seem to be related to a direct cytotoxic effect on tumour cells, as shown by lack of protection in normal mice challenged with serum-treated cells (Table 2). Moreover, the interference of *in vivo* tumour immunity was antigenically specific. Only the serum which contained antibodies against the appropriate TASA was effective. This result was similar to those obtained by testing the specificity by immunisation with various tumour cells or foetal cells¹², that is, *in vivo* protection was only obtained by immunisation with leukaemias induced by Friend, Moloney or Rauscher viruses (FRM specific), but not by immunisation with foetal tissue or tumour of other origin. These findings of the inability to interfere with antitumour immunity by anti-foetal serum provide another indication that foetal antigens in this system did not function as transplantation protection antigens. These data also supported the previous reports of the serologically detected TASA being a transplantation antigen^{9,12}.

This study has shown that only the specific antitumour antiserum can produce afferent interference of the *in vivo* tumour immunity. Thus, when well characterised, specific antisera are used, it can also help to test the specificity of TASA.

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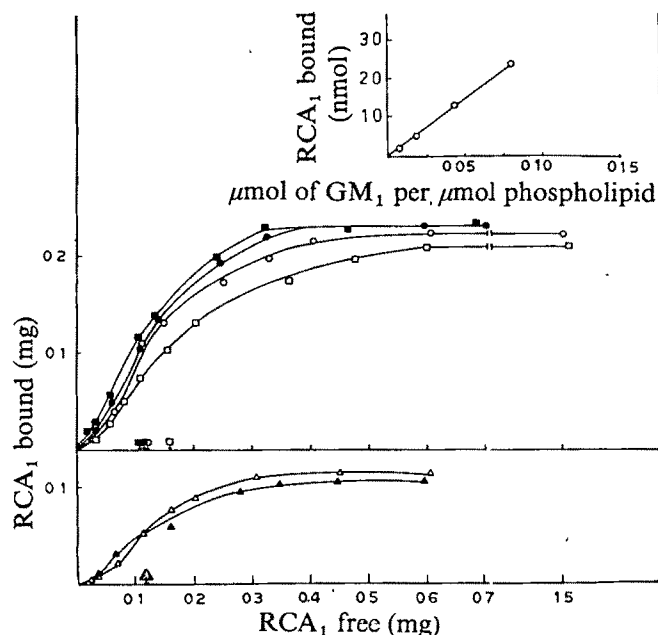
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Interaction between lectin from *Ricinus communis* and liposomes containing gangliosides

A MODEL has been presented showing the contribution of the protein-glycoprotein complex^{1–3} when transformed cells are agglutinated by lectins^{4–6}. But this has not been possible for the lectin-glycolipid complex because glycolipids form micelles, even at very low concentrations. This difficulty could be overcome by incorporating these glycolipids into lipid vesicles. (Haywood demonstrated that ganglioside incorporated in liposome can provide receptors for sendai virus⁷.) We have now studied the interaction between a galactose-specific lectin from *Ricinus communis* beans^{1,8,9} and liposomes containing different amounts of monosialoganglioside (GM₁), and found that the latter can be used as a model system for the investigation of agglutination of cells by lectins.

Gangliosides GM₁, GM₂, GD_{1a} and GT₁ (according to Svennerholm's nomenclature¹⁰) were isolated by thin-layer chromatography (TLC) from beef brain ganglioside prepared by the method of Folch *et al.*¹¹ Individual gangliosides were more than 90% pure as judged by TLC. *R. communis* lectin was purified as before¹ and lipid vesicles¹² were prepared by Huang's

Fig. 1 Binding of RCA₁ to liposomes of different ratios (*R*) of phospholipid to GM₁ at 4 °C. Various amounts of RCA₁ were added to a liposome suspension containing 6.4 nmol of GM₁ in 0.05 M phosphate buffer, pH 7.0, containing 0.15 M NaCl. The total volume of incubation medium was kept to 2 ml. Top, SC liposome: ○, *R* = 123; □, *R* = 55; ●, *R* = 22.8; ■, *R* = 12. Bottom, MC liposome: ▲, *R* = 147; △, *R* = 43.5. Inset, amount of RCA₁ bound as a function of ratio of GM₁ to phospholipid (1/*R*).



method¹³. Phospholipid (synthetic β - γ -dipalmitoyl-DL- α -lecithin, Sigma) and cholesterol (three times crystallised) in the molar ratio 2:1, with various concentrations of the different gangliosides (0.02–0.1 mol per mol of phospholipid) were dissolved in 2:1 chloroform-methanol. The solvent was subsequently removed under reduced pressure at 25 °C, and the flasks were placed in a vacuum desiccator for 4 h. The lipids were then suspended in 0.02 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and sonicated for 2 h under nitrogen at 4 °C. The liposomes thus obtained were centrifuged at 5,000g for 30 min to remove titanium particles and then subjected to gel filtration on Sepharose 4B to separate single compartment (SC) from multi-compartment (MC) liposomes described by Huang¹³. The fractions of SC and MC liposomes were pooled separately and concentrated to 1 ml. The amount of dipalmitoyl lecithin and ganglioside in the liposome was calculated by estimating lipid phosphorous¹⁴ and *n*-acetylneuraminic acid¹⁵, respectively. About 93% of phospholipid and 90–95% of gangliosides were incorporated in these liposomes. Thus liposomes containing GM₁, GM₂, GT₁ and GD_{1a} had been prepared.

Binding studies were done at 4 °C by adding various concentrations of lectin to the different batches of liposomes containing a constant amount of various glycolipids in 0.05 M sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl. The tubes were constantly shaken and after 3 h they were centrifuged at 105,000g for 2 h. The lectin remaining in the supernatant was estimated for protein by Lowry's method¹⁶ and subtracted from the amount added to give the amount bound to liposomes.

Liposomes without GM₁ and GM₂, GT₁ and GD_{1a} did not bind lectin whereas those containing GM₁ showed considerable binding (Fig. 1). Since all the lectin was recovered in the supernatant in the case of liposomes without GM₁, in the conditions of the binding experiment these way no significant entrainment of lectin. The association constant K_a (mol⁻¹) for the binding of incorporated GM₁ to lectin was calculated according to equation (1) from the value of m at $\theta = 0.5$.

$$K_a = \frac{\theta}{(1-\theta)m} \quad (1)$$

(where m = concentration of RCA₁ free in solution and θ = fraction of GM₁ at the outer surface in liposome, bound to RCA₁).

K_a thus calculated has a value of 2.2×10^6 mol⁻¹. Table 1 shows that about 27% and 60% of total GM₁ in liposome is available to bind to lectin at the outer surface of MC and SC liposomes, respectively. This agrees well with the amount of

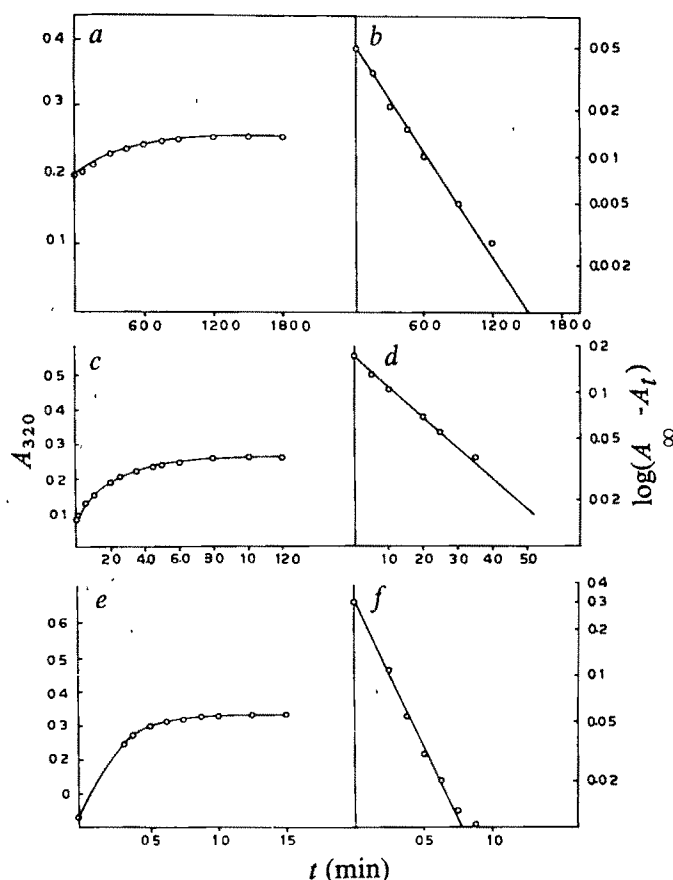


Fig. 2 Typical run for the kinetics of SC liposome aggregation on mixing with RCA₁ at 28 °C. Change in absorbance at 320 nm with time on mixing of RCA₁ and liposome containing GM₁ with different R values in 0.05 M phosphate buffer, pH 7.0, containing 0.15 M NaCl. *a*, $R = 123$; 3.5×10^{-7} mol of GM₁ in liposome and 2.3×10^{-6} mol of RCA₁. *c*, $R = 55$; 3.4×10^{-7} mol of GM₁ in liposome and 3.04×10^{-6} mol of RCA₁. *e*, $R = 22.8$; 2.37×10^{-7} mol of GM₁ in liposome and 1.66×10^{-6} mol of RCA₁. The corresponding semilog plots of *a*, *c* and *e* are presented in *b*, *d* and *f*, respectively.

n-acetylneuraminic acid liberated from GD_{1a} incorporated in liposome when treated with *Vibrio cholerae* neuraminidase (shown in parentheses in column 5 of Table). Binding of lectin to GM₁ liposome was prevented by lactose. The precipitin reaction between guar gum and lectin was not inhibited by GM₁, either below or above the critical micellar concentration¹⁷,

Table 1 Thermodynamic and kinetic parameters of the interaction between RCA₁ and liposomes with a different ratio (R) of phospholipid to GM₁ in 0.05 M phosphate buffer pH 7.0 containing 0.15 M NaCl

Type of liposome	Composition of liposome*	Amount of RCA ₁ bound (μg μmol ⁻¹ of phospholipid)	% of GM ₁ ‡ available for RCA ₁ binding	RCA ₁ binding sites per μm ² §	Association constant (K_a (4 °C) $\times 10^{-6}$ mol ⁻¹)	Rate of formation of clusters (k_f (mol ⁻¹ s ⁻¹) at 28 °C $\times 10^{-9}$)
	Phospholipid (μmol ml ⁻¹)	GM ₁ † (μmol ml ⁻¹)		$\times 10^{-3}$		
SC	10.5	0.085 (123)	264.0	55 (65 ± 5%)	1.55	2.04
SC	10.0	0.185 (55)	635.0	56 (65 ± 6%)	4.10	1.52
SC	10.8	0.475 (22.8)	1,560.0	60 (—)	10.40	2.18
SC	10.5	0.87 (12)	2,886.0	58 (—)	19.0	2.22
MC	12.5	0.085 (147)	110.0	25 (—)	—	2.20
MC	12.0	0.275 (43.5)	403.0	27 (30 ± 3%)	—	2.20

The results presented in columns 5, 6, 7 and 8 are the average of three independent experiments.

*Dipalmitoyl lecithin and ganglioside (GM₁) in liposome was calculated by estimating lipid phosphorous¹⁴ and *n*-acetylneuraminic acid¹⁵, respectively.

†Numbers in parentheses indicate values of R (phospholipid per GM₁).

‡GM₁ available at the surface was calculated using a saturation value for RCA₁ considering RCA₁ bivalent

This was confirmed by preparing GD_{1a} liposome and subjecting it to *Vibrio cholerae* neuraminidase.

§The number of molecules of *n*-acetylneuraminic acid liberated from these liposomes corresponds to the amounts of GD_{1a} molecules accessible to hydrolysis by neuraminidase (that is exposed on the outer surface of liposome). This number is indicated in parentheses under ‡.

§Based on the molecular weight of SC liposome as 2×10^6 daltons and its diameter as 300 Å (ref. 13).

¶For a 1-ml reaction mixture containing 1.73×10^{-7} mol of GM₁ and 3×10^{-6} mol of RCA₁.

suggesting that there was no binding of free GM₁ with lectin.

When lectin was added to liposome containing GM₁, the absorbance (that is turbidity) at 320 nm increased with time presumably due to the formation of liposome clusters which resemble cell agglutination. The rate at which turbidity increased at 320 nm was dependent on the composition of the liposome, that is on the number of lectin binding sites per μm^2 , although K_a remained constant. Figure 2 shows the typical kinetic run for SC liposomes with different ratios of phospholipid to GM₁ (R values), carried out with lectin in excess GM₁ available at the outer surface (pseudo-first order conditions). From the slope of plot of $\log(A_\infty - A_t)$ against t (where A_t and A_∞ are the absorbance at time t and infinity) the pseudo-first order rate constant k_{obs} is calculated. There from the second-order rate constant of cluster formation, $k_f = (k_{obs}/C)$, where C is the concentration of lectin in mol^{-1} based on a molecular weight of lectin of 120,000) are summarised in Table 1. It is interesting that a factor of two increase in available binding sites for lectin leads to an increase in the rate constant of formation by a factor of 20. The k_f value for GM₁ liposome ($R = 55$) is comparable with that for the glycoprotein-concanavalin A (Con A) system¹, whereas k_f for the liposome ($R = 22.8$) is comparable with that for the 'precipitin' reactions between glycogen and con A¹ and between guar gum and *R. communis* lectin. The latter values compare well with the rate constant of $8 \times 10^4 \text{ mol}^{-1} \text{ s}^{-1}$ for the formation of the soluble con A-carbohydrate complex, reported by Gray and Glew¹⁸.

From our measurements we draw the following conclusions. (1) The incorporated gangliosides are recognised through the non-reducing terminal galactose moiety; this is consistent with our studies with galactose-containing glycoproteins¹⁹. (2) The K_a of this interaction is 100–1,000 times greater than those of galactose and *p*-nitrophenyl- β -D-galactopyranoside, respectively^{1,9}. This value may arise from any of the following and/or any combination of (i) interaction of protein component with the lipid matrix, (ii) different conformations of carbohydrates of the glycolipid when embedded in lipid phase and when free in solution, (iii) alteration of the polarity of the intervening medium due to the presence of the glycolipid cluster. Any differences in these factors may lead to slight differences in K_a values calculated from the 50% saturation values. (3) The rate constant of cluster formation is significantly altered depending on the number of sites per μm^2 of liposome surface which are likely to be uniformly distributed, whether they are MC or SC. This suggests that GM₁ in liposome which are available to bind with lectin with more or less the same affinity are kinetically distinguishable when the ratios of GM₁ and phospholipid in the liposome are varied. The reason for this is not clear. It may be because GM₁ is differently located in the lipid matrix or because surface diffusion controls the rate of formation of clusters, as measured by increased turbidity. Nevertheless our findings are consistent with the idea that the observed increased agglutinability of transformed cells is mediated through (1) change in the topological distribution of receptor site and (2) membrane fluidity. Further studies are required to assess quantitatively how the membrane fluidity (determined by the nature and composition of lipid matrices) influences the kinetic behaviour of cluster formation representing cell agglutination.

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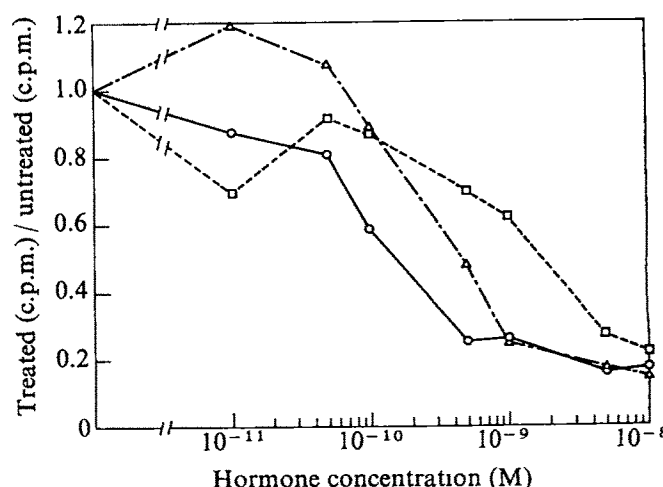
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Differential effects of glucocorticoids on DNA synthesis in normal and virus-transformed chick embryo cells

Loose connective tissue fibroblasts in the mouse become markedly non-motile and refractile and lose pinocytotic activity within about an hour of hydrocortisone infusion. Only minutes are required to mimic this response in culture¹. These responses of fibroblasts are well correlated with the topical anti-inflammatory potency of the glucocorticoids². Glucocorticoids inhibit the multiplication in primary cultures of rat and mouse fibroblasts and of human epithelial cells^{3–6}. In marked contrast, they stimulate multiplication of 3T3 cells⁷. Some investigators regard the 3T3 cell as a prototype for the study of growth regulation in

Fig. 1 Inhibition by glucocorticoids of DNA synthesis in cultures of chick embryo cells. 10^6 chick embryo cells were seeded in 60-mm Petri dishes. After 3 d growth, the medium was changed to one without serum, and one of three glucocorticoids was added at the indicated concentrations for 16 h. The rate of ^3H -TdR incorporation into DNA for 1 h was measured by extraction and scintillation counting, and the total protein content was measured¹². \circ , Dexamethasone; Δ , hydrocortisone; \square , cortisone. We used published cell and virus culturing techniques^{9–11}. DNA synthesis was measured by the incorporation of ^3H -thymidine (^3H -TdR) into extractable DNA, or by autoradiography¹². Protein was determined by the Lowry method for cells in culture¹². Glucose transport was measured by the uptake of ^3H -2-deoxy-D-glucose (^3H -2dGlc)¹². In several preliminary experiments, we observed that glucocorticoids did not affect DNA synthesis in chick embryo cultures if the hormones were added to medium conditioned by one or more days of exposure to growing cells. We therefore carried out all experiments with cultures that received fresh changes of medium.



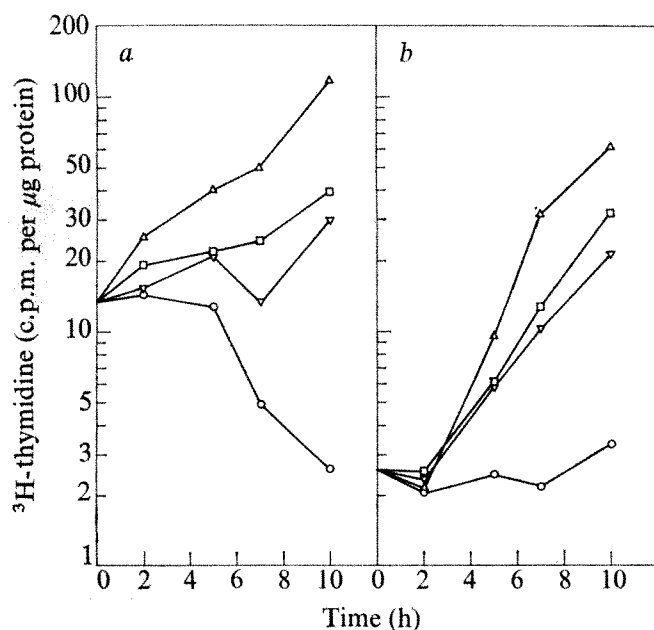


Fig. 2 Kinetics of inhibition and restoration of DNA synthesis in chick embryo cultures after hydrocortisone treatment and removal. Cultures of chick embryo cells were treated for 16 h with serum-free medium (a) or with serum-free medium containing 10^{-7} M hydrocortisone (b). The following media then were substituted in both groups: Δ , 1% serum, no hydrocortisone; \square , 1% serum, with hydrocortisone; ∇ , no serum, no hydrocortisone; \circ , no serum, with hydrocortisone. At the indicated times, the incorporation for 1 h of ^3H -TdR into DNA was determined.

cultured fibroblasts, although this line has been identified as tumorigenic in some conditions⁸.

We have determined the effects of glucocorticoids on primary chick embryo fibroblast cultures in which a variety of growth-regulatory parameters were measured, and on the same cells after they had undergone virus-induced malignant transformation. We found that the glucocorticoids in very low concentrations retarded the progress of normal chick embryo fibroblasts through the G_1 period of the cell cycle, thereby reducing the fraction of cells synthesising DNA at any given time. The same concentrations of glucocorticoids had no effect on the initiation of DNA synthesis in virus-transformed cultures.

Figure 1 shows the effects of treatment for 16 h with each of three glucocorticoids on DNA synthesis in normal chick embryo cells. (We chose 16 h after hormone treatment as the time for measuring maximum inhibition of DNA synthesis, as shown in Fig. 3.) All three glucocorticoids reduced ^3H -TdR incorporation into DNA about four- to sixfold. Levels of 1.5×10^{-10} M dexamethasone, 4.5×10^{-10} M hydrocortisone and 1.5×10^{-9} M corticosterone caused 50% inhibition of DNA synthesis. These levels are all within the physiological concentration range of glucocorticoids¹³.

We measured the kinetics of inhibition and restoration of DNA synthesis by hydrocortisone in the presence or absence of serum. Inhibition of DNA synthesis definitely was evident both in the presence and absence of serum within 4 h of administration of the hormone, and inhibition became more pronounced with time up to 10 h (Fig. 2a). Similarly, increased DNA synthesis was apparent within 4 h of removal of hydrocortisone and became more pronounced with time up to 10 h (Fig. 2b). Inhibition by the hormone of the rate of incorporation of ^3H -TdR into extractable DNA was proportional to reduction in the fraction of nuclei labelled with ^3H -TdR, as detected by autoradiography (Fig. 3).

The uptake of ^3H -2-dGlc into uninfected cells was inhibited more rapidly than was the incorporation of ^3H -TdR

into DNA, but the extent of inhibition at 16 and 24 h was about the same for both substances (Fig. 4). Hydrocortisone in concentrations as high as 1.0×10^{-5} M had no effect on DNA synthesis in cultures of chick embryo fibroblasts transformed by infection with Rous sarcoma virus (Table 1).

These data indicate that the growth of normal fibroblasts in primary culture is inhibited by glucocorticoids, just as it is in the animal. This result contrasts markedly with the stimulatory effect of glucocorticoids on the growth of the 3T3 cell line⁷, which has been widely accepted as the prototype of normalcy for fibroblasts in culture. The demonstration that 3T3 cells produced haemangioendotheliomas in mice in certain conditions⁸ raises serious questions about the use of 3T3 cells for studying growth-regulatory processes. These doubts are accentuated by the paradoxical response of the cells to glucocorticoids.

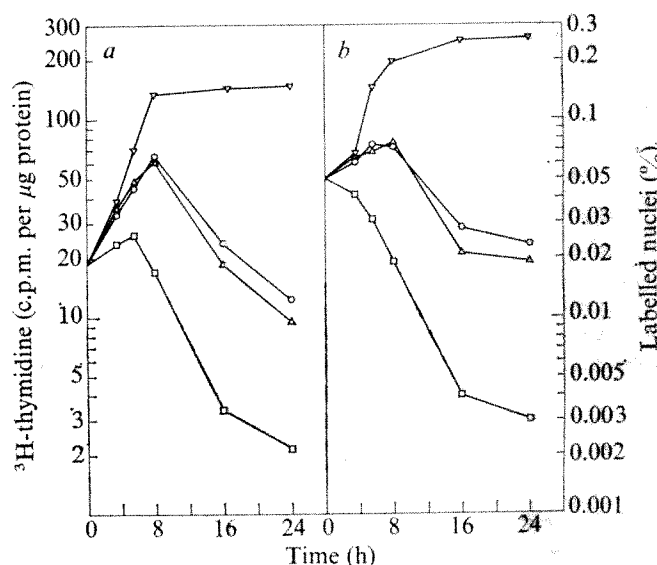


Fig. 3 Comparison of scintillation counting and autoradiography in measuring effects on DNA synthesis with time after hydrocortisone treatment. Cultures of chick embryo cells were treated for various times with 10^{-7} M hydrocortisone in the presence or absence of 1% serum. ^3H -TdR incorporation into DNA was measured by extraction and scintillation counting (a), and by autoradiography (b). ∇ , 1% serum, no hydrocortisone; Δ , 1% serum, with hydrocortisone; \circ , no serum, no hydrocortisone; \square , no serum, with hydrocortisone.

We have shown that the inhibition of growth by glucocorticoids is associated with a reduction in the number of cells synthesising DNA that is proportional to the reduction in ^3H -TdR incorporation into extractable DNA. This indicates that glucocorticoids have no effect on DNA chain elongation and act by slowing the progress of cells through the G_1 period of the cell cycle. Thus, the effect is similar to that produced by increasing population density or by

Table 1 Comparative effect of hydrocortisone on normal and virus-transformed chick embryo cells

Hydrocortisone (M)	^3H -thymidine (c.p.m. per μg protein)* Uninfected	^3H -thymidine (c.p.m. per μg protein)* Transformed
Control	13.54	101.70
10^{-7}	2.95	117.20
10^{-6}	3.01	112.46
10^{-5}	3.05	103.44
10^{-4}	2.75	30.46

*Average of duplicates 16 h after initiation of treatment. Cultures were prepared as described in Fig. 1, and half were infected with the Schmidt-Ruppin strain of Rous sarcoma virus (1×10^6 focus-forming units). The medium was changed on both sets of cultures after 36 h and again on day 3. The latter change of medium contained the indicated hydrocortisone concentrations. ^3H -TdR incorporation into DNA was measured 16 h after hormone treatment.

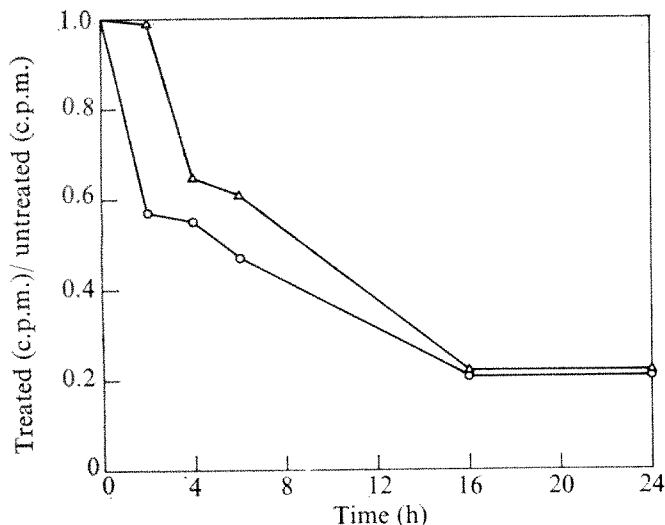


Fig. 4 Kinetics of inhibition by hydrocortisone of $^3\text{H-2-dGlc}$ uptake and $^3\text{H-TdR}$ incorporation. The medium of 3-d-old chick embryo cultures was changed to one without serum, and 10^{-7} M hydrocortisone was added to half the cultures. At the indicated times, measurements were made of $^3\text{H-2-dGlc}$ uptake into acid-soluble material and of the incorporation of $^3\text{H-TdR}$ into acid-insoluble material. Protein content was determined by the Lowry method. ○, $^3\text{H-2-dGlc}$; △, $^3\text{H-TdR}$.

lowering either serum concentration or pH (ref. 11). Glucocorticoids produce the same inhibitory effect as these treatments on the uptake of $^3\text{H-2-dGlc}$ and have similar rapid kinetics.

Overall, these results suggest that inhibitory treatments act through a common mechanism related to the hypothetical mechanism by which diverse growth-stimulatory agents act^{14,15}. In the latter hypothesis, the coordinated control of many processes in chick embryo cells is effected through regulation of the availability of divalent cations for enzymatic reactions and other cellular functions¹⁵. The most important of the cations for metabolic control is Mg^{2+} , because of its essential role in transphosphorylation reactions which are the regulatory steps in many biochemical pathways. A mechanism for control of divalent cation activity is suggested by the observation that the stimulatory hormone insulin, decreases the binding of Ca^{2+} to plasma membranes, whereas hydrocortisone increases that binding¹⁶. Substances that modulate Ca^{2+} binding to membranes are also likely to affect Mg^{2+} binding as well, as both cations have similar binding properties for membranes¹⁷. The availability of Mg^{2+} for metabolic processes depends on its partitioning between free and membrane bound forms¹⁷.

Hydrocortisone failed to inhibit DNA synthesis in virus-transformed cultures at a concentration almost 10^5 times higher than that which produces 50% inhibition in normal cells. This observation supports the claim that tumour cells lose their sensitivity to the insulin-anti-insulin set of controls found in normal cells¹⁸. The regulatory mechanism proposed above suggests that membranes of tumour cells have diminished capacity for binding divalent cations and that this characteristic cannot be modified by glucocorticoids. Information on whether this is an intrinsic defect of the membrane or a response conditioned by the internal cell milieu should shed some light on the mechanism of carcinogenesis.

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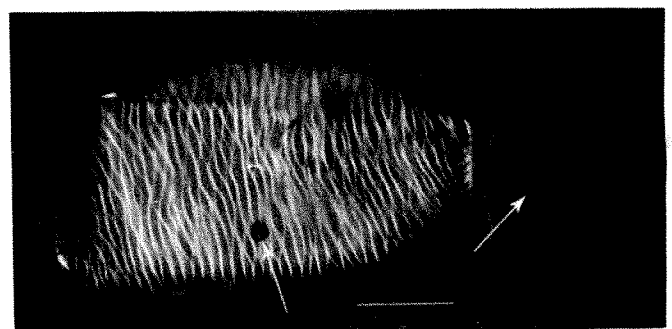
Differentiation of wound vessel members without DNA synthesis, mitosis or cell division

THE formulation of a general model of gene regulation in eukaryotic organisms would be facilitated were it known when the molecular events responsible for reprogramming of genetic information occur during the cell cycle. Evidence from various systems in which animal cells differentiate *in vivo* and *in vitro* suggests that certain characteristics of the fully differentiated state are not manifest until the cells undergo DNA synthesis and nuclear division¹. Similarly the differentiation of wound vessel members (WVM) in plant parenchyma tissue is thought to occur only after cells have replicated at least once in inductive conditions².

WVM are highly specialised elements of the xylem which differentiate in parenchyma tissue as a response to wounding. They are characterised by the deposition of a patterned cellulose secondary wall which becomes lignified and remains as a resistant structure when the cell contents undergo autolysis at maturity (Fig. 1). Dalessandro and Roberts³ observed WVM formation when caffeine was used to inhibit cytokinesis. They considered their results to be inconclusive, however. Other references^{4,5} have been made to WVM formation without cell division but these reports have been based only on the relative sizes of WVM and surrounding undivided parenchyma cells.

I report here the differentiation of WVM without DNA synthesis, mitosis or cell division in cultured explants of Romaine lettuce (*Lactuca sativa* L. cv Romana). Cylindrical disks of parenchyma tissue, 5 mm in diameter and 1.5 mm thick, were isolated aseptically from the pith of Romaine lettuce heads and placed basal end down on a chemically defined medium⁶ containing the DNA synthesis inhibitor fluorodeoxyuridine (FUDR) (10^{-6}M). The numbers of WVM and parenchyma cells were assayed after 14 d of incubation.

Fig. 1 Immature WVM and parenchyma cell in Feulgen-stained squash preparation. Photographed with partially polarised illumination to highlight the birefringent secondary wall of the WVM. Both cells are nucleate (arrows). Bar represents 50 μm .



The results of a representative experiment are presented in Table 1. Disks placed on a medium without FUDR underwent extensive proliferation, as expected, producing approximately 25 times the original number of cells, 7% of the final cell number being WVM. In disks grown on medium with FUDR 6% of the cells differentiated into WVM without any apparent increase in cell number.

There was considerable variation in the number of WVM produced in the presence of FUDR; the average number per disk ranging between 0 and 9×10^3 . This variation was probably due to seasonal effects and to the commercial origin of the plant material. Results were, however, consistent within single experiments.

Table 1 Parenchyma cells and wound vessel members in tissue disks incubated with or without FUDR

	Parenchyma cells ($\times 10^3$)	Wound vessel members ($\times 10^3$)
0 d	87.9 ± 5.6	0
14 d (no FUDR)	$2,240 \pm 220$	180 ± 13
14 d (10^{-5} M FUDR)	79.0 ± 3.8	4.7 ± 1.2

Parenchyma cells and WVM in tissue disks incubated with or without FUDR. Heads of Romaine lettuce were purchased at a local retail outlet, defoliated and surface-sterilised with 0.5% sodium hypochlorite. Cylinders of tissue 5.0 mm in diameter were removed from the centre of the core with a sterile cork borer and sliced into disks 1.5 mm thick. Disks were placed with their morphological basal end down on sterile medium⁶ containing indole-3-acetic acid (10 mg l^{-1}), kinetin (6-furfurylaminopurine, 0.1 mg l^{-1}) and 0.9% rehydrated Difco agar in 6-cm diameter Petri plates. FUDR was filter-sterilised and added to the medium when it had partially cooled. The tissue disks were cultured in the dark at $25 \pm 1^\circ \text{C}$ and at the end of the incubation period dispersed in chromic acid-HCl solution by drawing through a 22-gauge needle⁶. Cell counts were made in six 5- μl samples of an appropriate dilution of the macerate placed on a microscope slide.

Parenchyma cells of the pith are, *in situ*, quiescent and DNA synthesis does not begin for at least 20 h after isolation of the explants (unpublished). Although this seemed more than enough time for the uptake of FUDR in quantities sufficient to inhibit DNA synthesis, the effectiveness of the inhibitor was tested directly by microspectrophotometric measurement of the DNA content of Feulgen-stained nuclei (Fig. 2).

All nuclei of freshly isolated pith contained the 2C level of DNA, confirming earlier reports^{7,8} that cells of this species arrest exclusively in the G_1 phase of the nuclear cycle. In the nuclei of cells grown for 14 d on medium without FUDR the DNA content ranged between 2C and 4C. In FUDR-treated disks, however, there was no measurable DNA synthesis in the nuclei of parenchyma cells. The DNA content of nuclei in 50 immature WVM which had not yet undergone autolysis was also measured. These cells were unambiguously identified as WVM by the presence of secondary walls in the characteristic reticulate pattern (Fig. 1). The amount of nuclear DNA in WVM which had just begun to differentiate corresponded to the 2C level. As these cells continued to differentiate chromatin condensed, leading to an increase in self-absorption and slightly lower values of relative DNA content.

Mitosis and cell division could not have occurred in FUDR-treated disks since all cells were originally in G_1 and did not synthesise measurable amounts of DNA. To verify this conclusion, freshly isolated disks were placed on medium containing FUDR (10^{-5} M) and removed at 12 h intervals for the next 14 d. The disks were stained with Feulgen and squash preparations were systematically and thoroughly examined. WVM were first detected after 5 d of incubation and reached a maximum of approximately 2×10^3 per disk by day 12. No mitotic figures were found in any of the preparations.

The Romaine lettuce-xylogensis system is particularly significant in that the cells which differentiate are apparently uncommitted to the formation of vessel members before isolation. The pith tissue of this species is composed of a

homogeneous population of parenchyma cells devoid of vascular tissue. Xylem elements form only as a response to injury and do not normally differentiate in the pith during the life cycle of an undisturbed plant. In seedlings of wheat germinated from gamma-irradiated grain, differentiation of complex tissues, including xylem, takes place without DNA synthesis⁹. But vessels arise only in procambial tissue already formed in the embryo and presumably committed to differentiation¹⁰. Similarly, there have been reports that, in appropriate conditions, determined mammalian¹¹ and insect^{12,13} cells may differentiate without DNA replication.

Evidence linking differentiation to a previous round of cell division has often led to the suggestion that events during the cell cycle, especially in the DNA replication phase, are themselves integral components of a genetic reprogramming mechanism (reviewed in ref. 14). While most plant and animal cell differentiation undoubtedly occurs after cell division, the experimental uncoupling of these processes suggests that this is not a causal association. In the meristem, cell doubling may be an added constraint on differentiation to ensure that progenitor cells are reproduced before differentiation occurs. A homeostatic mechanism of this nature would protect the integrity of the progenitor cell line *in vivo* and may be responsible for the frequently observed coincidence of cell division and

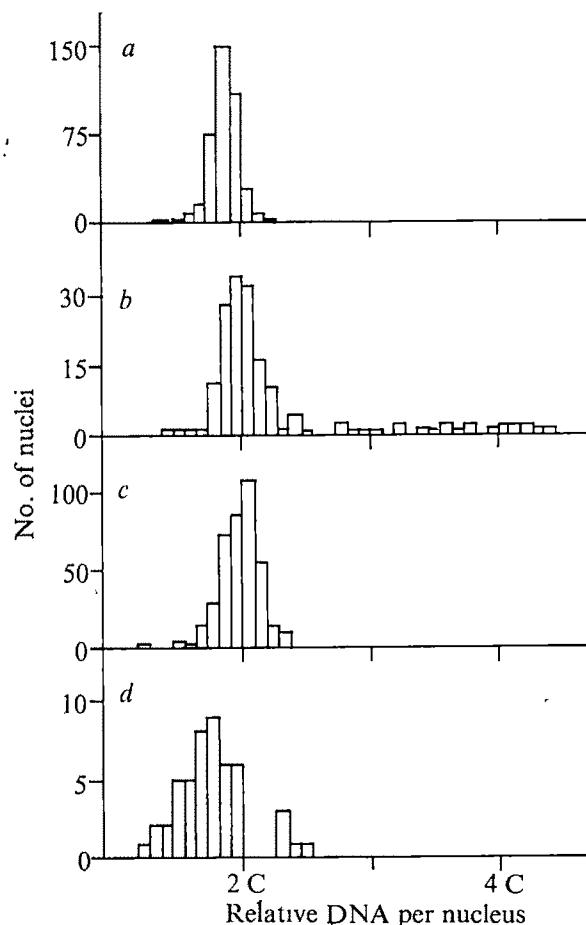


Fig. 2 Relative amounts of DNA per nucleus before and after 14 d of incubation (Table 1). *a*, Freshly isolated parenchyma cells, sample size (N) = 400. *b*, Parenchyma cells after 14 d incubation no FUDR in medium, N = 165. *c*, Parenchyma cells after 14 d incubation, 10^{-5} M FUDR, N = 400. *d*, Immature WVM after 14 d incubation, 10^{-5} M FUDR, N = 50. Disks of tissue were fixed in ethanol-acetic acid (3:1 v/v), hydrolysed in 5N HCl for 60 min at room temperature and stained by the Feulgen method. The stained preparations were placed in a 5% pectinase solution for 2 h and drawn through a 22-gauge needle to separate cells before mounting in Canada balsam. Measurements of relative DNA per nucleus were made with a Zeiss scanning microspectrophotometer at 560 nm and normalised with readings of half telophase and prophase mitotic figures taken to be 2C and 4C values, respectively.

differentiation *in vitro*. Such a mechanism might also operate where animal cells differentiate in proliferating tissue.

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Stimulation of phosphorylation and inactivation of pyruvate dehydrogenase by physiological inhibitors of the pyruvate dehydrogenase reaction

OXIDATION of pyruvate in perfused rat heart is inhibited by diabetes and by oxidation of fatty acids and ketone bodies¹. Enhanced release and oxidation of fatty acids from muscle glycerides may be an important factor in the effects of diabetes²⁻⁴. These effects on pyruvate oxidation have generally been attributed to inhibition of pyruvate dehydrogenase, and regulation of this enzyme is considered to be of crucial importance in the selection of fuels for respiration and in the metabolic changes in heart and other tissues in diabetes and starvation.

Mammalian pyruvate dehydrogenases contain three enzymes, pyruvate decarboxylase (E_1), dihydrolipoate acetyl

transferase (E_2) and dihydrolipoate dehydrogenase (E_3) bound together in a single complex. They catalyse the conversion of pyruvate, CoA and NAD into acetyl CoA, NADH₂ and CO₂ in the presence of Mg²⁺ and thiamine pyrophosphate (TPP) by a coordinated series of reactions in which lipoate bound covalently to the acetyl transferase visits active sites on the three enzymes sequentially. Lipoate reacts with hydroxyethyl TPP formed on E_1 to give acetyl hydrodipoate which reacts with CoA on E_2 to form acetyl CoA and dihydrolipoate, the latter then reacts with NAD on E_3 to form NADH₂ and regenerate lipoate⁵. Reversible reactions are catalysed by E_2 and E_3 , whereas the reaction catalysed by E_1 is essentially irreversible. NADH₂ can thus form dihydrolipoate, and acetyl CoA plus NADH₂ can form acetyl hydrodipoate and hydroxyethyl TPP within the complex by reversal of reactions catalysed by E_2 and E_3 .

Pyruvate dehydrogenase in heart muscle or liver is subject to end product inhibition by acetyl CoA (competitive with CoA) and NADH₂ (competitive with NAD) and this inhibition is presumed to be due to accumulation of acetyl hydrodipoate and dihydrolipoate within the complex^{6,7}. Mammalian pyruvate dehydrogenases are also regulated by pyruvate dehydrogenase kinase within the complex, which catalyses phosphorylation and inactivation of pyruvate decarboxylase with ATP-Mg²⁺; a phosphatase catalyses dephosphorylation and reactivation^{8,9}. In perfused heart oxidation of fatty acids or ketone bodies leads to a substantial increase in the concentration ratio of acetyl CoA-CoA. With acetate, for example, this ratio increases sixtyfold and oxidation of pyruvate is almost totally suppressed within 1 min of entry of acetate into the coronary circulation^{10,11}. It has been suggested that this change in concentration ratio may lead to end product inhibition of pyruvate dehydrogenase and thus contribute to inhibition of pyruvate oxidation. In perfused heart oxidation of fatty acids and ketone bodies also stimulates phosphorylation and inactivation of pyruvate dehydrogenase^{12,13}. The mechanism of this effect is not known but it presumably involves activation of the kinase reaction or inhibition of the phosphatase reaction or both.

The known effectors of the kinase reaction (ADP, pyruvate, pyrophosphate compounds, Ca²⁺ and Mg²⁺ as inhibitors) and of the phosphatase reaction (Mg²⁺ and Ca²⁺ as activators^{8,9,14,15}) did not provide an obvious explanation of effects of fatty acids and ketone bodies in the perfused

Table 1 Effects of NAD, NADH₂, CoA and acetyl CoA on activity of pig heart pyruvate dehydrogenase kinase

Concentration (mM)				Pyruvate dehydrogenase kinase activity (mean \pm s.e., counts per 10 min in protein-bound phosphate)	
NAD	NADH ₂	CoA	acetyl CoA		
0	0	0	0	3,812 \pm 47 (12)	Effect of NAD, NADH ₂ and ratio of NADH ₂ -NAD
2	0	0	0	3,753 \pm 42 (3)	
0	0.01	0	0	4,853 \pm 104 (3)	
2	0.01	0	0	3,958 \pm 26 (3)	
0	0.5	0	0	4,830 \pm 91 (3)	
2	0.5	0	0	4,113 \pm 96 (3)	Effect of CoA
2	0.01	0	0	3,958 \pm 26 (3)	
2	0.01	0.25	0	2,922 \pm 51 (3)	
2	0.01	2.0	0	2,148 \pm 35 (3)	
2	0.5	0	0	4,113 \pm 96 (3)	
2	0.5	0.25	0	3,518 \pm 66 (3)	Effect of acetyl CoA
2	0.5	2.0	0	2,593 \pm 26 (3)	
2	0.01	0	0	3,958 \pm 26 (3)	
2	0.01	0	0.25	4,561 \pm 41 (3)	
2	0.5	0	0	4,113 \pm 96 (3)	
2	0.5	0	0.25	4,613 \pm 17 (3)	Effect of ratio of acetyl CoA-CoA
2	0.5	2.0	0	2,593 \pm 26 (3)	
2	0.5	2.0	0.25	3,884 \pm 82 (3)	
2	0.5	2.0	0.5	4,282 \pm 98 (3)	
2	0.5	2.0	1.0	4,607 \pm 54 (3)	

Pig heart pyruvate dehydrogenase (2 U ml⁻¹) was incubated at 30 °C for 1.5 min in 20 mM potassium phosphate, 5 mM 2-mercaptoethanol, pH 7.0 (volume 50 μ l) with γ -³²P-ATP (100 μ M, 8 μ Ci μ mol⁻¹) and 2 mM MgCl₂ with other additions as shown. The reaction was terminated and protein-bound phosphate assayed in 30 μ l samples as in ref. 15. The number of assays is shown in parentheses.

heart. It seemed likely that the concentration ratios of acetyl CoA-CoA and of NADH₂-NAD might modulate kinase or phosphatase reactions in addition to their effects on the dehydrogenase reaction. No consistent and significant effects of NAD, NADH₂, acetyl CoA or of CoA on the phosphatase reaction¹⁶ have been detected. Attempts to demonstrate effects of the concentration ratio of acetyl CoA-CoA on the kinase reaction yielded very variable results¹⁵. Recent studies of elementary reactions in the pyruvate dehydrogenase complex suggested that the concentration ratio of NADH₂-NAD might be critical to a demonstration of effects of the concentration ratio of acetyl CoA-CoA on the kinase reaction.

This expectation has been realised in the present study in which pyruvate dehydrogenase kinase activity has been measured by the incorporation of ³²P into protein-bound phosphate from γ -³²P-ATP by an assay procedure described previously¹⁵. The only variation in the present study has been addition of effectors immediately before initiation of the reaction with γ -³²P-ATP. As shown by representative data in Table 1, NADH₂ activates the kinase reaction and NAD reverses the NADH₂ activation. In the presence of mixtures of NADH₂ and NAD, which themselves only partially activate, acetyl CoA activates the kinase reaction and CoA inhibits. Acetyl CoA reverses the inhibition by CoA provided sufficient NADH₂ is present. The pyruvate dehydrogenase kinase reaction is thus sensitive to concentration ratios of NADH₂-NAD and of acetyl CoA-CoA in a manner which may provide a basis for effects of fatty acids and ketone bodies also stimulates phosphorylation and inactivation of pyruvate dehydrogenase in perfused heart.

The mechanism of these complex effects of NADH₂, NAD, CoA and acetyl CoA is not known. In pyruvate dehydrogenase, E₂ forms the core of the molecule and carries E₁, E₃ and pyruvate dehydrogenase kinase¹⁷. If NADH₂, NAD, acetyl CoA and CoA affect the kinase reaction through binding to their substrate sites on E₂ and E₃, their effects must be transmitted from these subunits to the kinase or its substrate (E₁). One possibility is that lipoate might transmit these effects because it visits active sites on E₁, E₂ and E₃; the presence of oxidised lipoate on E₁ might inhibit phosphorylation of E₁ by the kinase so that when lipoate in one of its forms is located on E₂ or E₃ the kinase reaction could be facilitated. More conventional explanations for regulatory interactions between subunits involving either binding of the effectors to their substrate sites on E₂ and E₃ or to additional regulator sites on E₁ or the kinase may provide an alternative explanation for these effects of NADH₂, NAD, acetyl CoA and CoA.

Since this manuscript was submitted, Pettit *et al.*¹⁸ have reported inhibition of beef heart pyruvate dehydrogenase kinase reaction by CoA and by NAD, and activation of the kinase reaction by NADH₂ and by acetyl CoA. Our results with pig heart complex provide confirmation for inhibition by CoA and activation by NADH₂. We have not detected inhibition by NAD but rather reversal of NADH₂ activation by NAD. As mentioned earlier, we have not been able to detect significant activation of the kinase reaction by acetyl CoA except in the presence of mixtures of NAD plus NADH₂ which themselves produce no or only partial activation of the kinase reaction.

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Pregnancy in the hyrax and erythrocyte metabolism of progesterone

HYRAXES are unusual for small mammals because they have a long gestation period (7.5 months) for their size (2-3 kg) and intra-abdominal testes^{1,2}. Hystricomorph rodents with long gestation periods relative to mature weight have a pregnancy-specific plasma protein with a high affinity for progesterone—progesterone-binding globulin (PBG). It occurs at high concentrations in the guinea pig and coypu, and evidently reduces the rate of progesterone clearance from blood³. We have found that, in the hyrax, the progesterone concentration in the corpus luteum is appreciable whereas that in plasma is low, and that erythrocytes metabolise progesterone *in vitro*. The results of this study are relevant to the phylogenetic affinity of the hyrax and elephant, and there is doubt about whether progesterone is the hormone of pregnancy in the elephant, since its concentration in the corpus luteum and peripheral plasma has been found to be very low⁴⁻⁷.

Steroid hormones of pregnancy were measured in the rock hyraxes, *Heterohyrax brucei* and *Procavia habessinica*, shot near Nairobi, and in captive *H. brucei*. Blood of shot specimens was taken into heparinised syringes and the ovaries and placentae removed immediately; all samples were transported to the

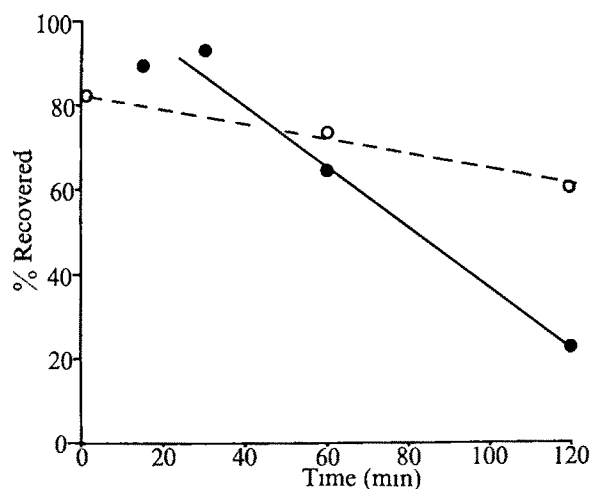


Fig. 1 The metabolism and uptake of ³H-progesterone by hyrax blood. Whole blood (0.5 ml) was incubated with ³H-progesterone, 0.5 μ Ci, 2.8 ng, at 4 °C (○) and 37 °C (●). Labelled progesterone was extracted from blood with 5 ml diethyl ether after the addition of 100 μ g unlabelled steroid to correct for procedural losses. After extraction, progesterone was separated by thin-layer chromatography (75:25, benzene-ethyl acetate), eluted with ethanol, and its specific activity determined by ultraviolet spectrophotometry and liquid scintillation spectrometry. The amount of labelled progesterone recovered was corrected for procedural losses and the result expressed as a percentage of added ³H-progesterone. The metabolism of ³H-progesterone at 4 °C was negligible and the apparent loss of steroid was related to red cell uptake. Enzymatic metabolism at 37 °C, however, was substantial.

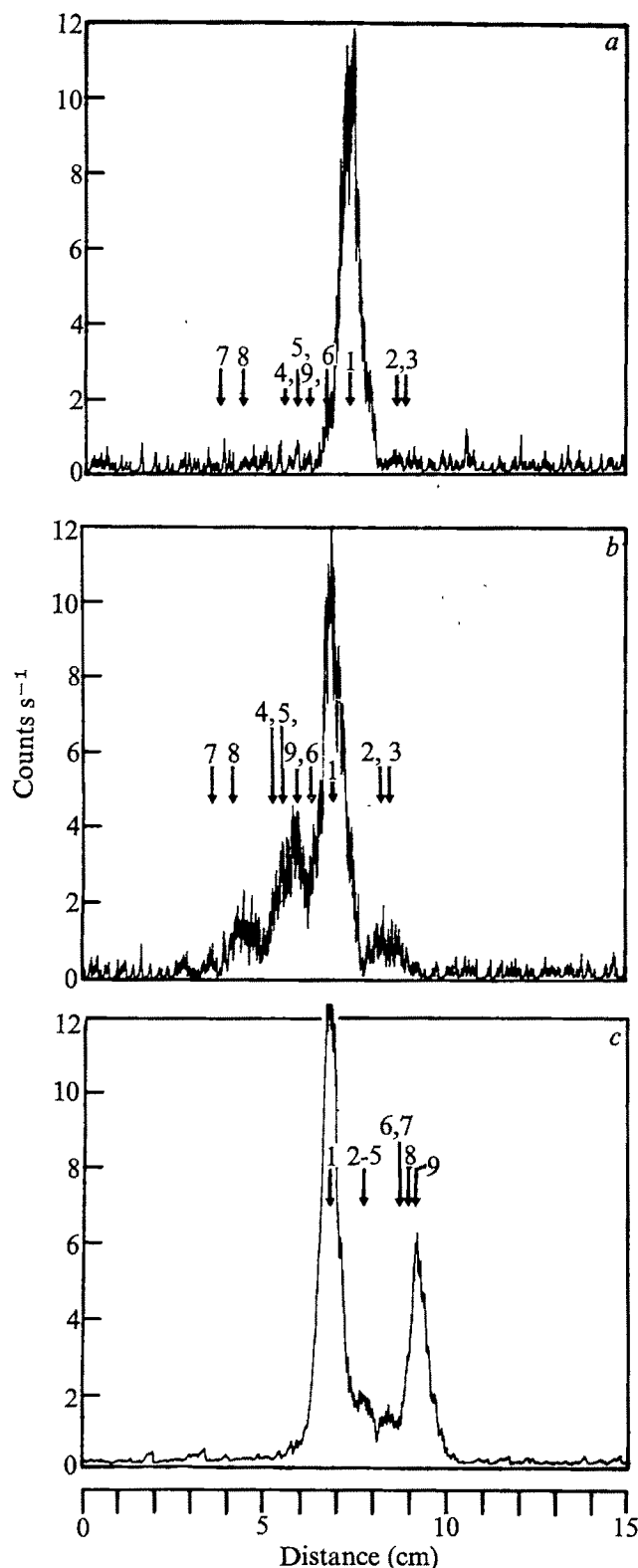


Fig. 2 The metabolism of ^3H -progesterone by hyrax blood. Saline or whole blood (5 ml) was incubated with $2.5 \mu\text{Ci}$ ^3H -progesterone (14 ng) at 37°C for 3 h. After incubation, 1 ml 2.5% NaOH was added and each sample was extracted with 25 ml diethyl ether which was washed with distilled water and evaporated to dryness. The samples were applied to thin-layer chromatography plates and run for 15 cm in the solvent system, benzene-ethyl acetate, 4:6. a, ^3H -Progesterone added to saline, b, ^3H -progesterone added to hyrax blood; c, ^3H -compounds recovered from plate b acetylated at 70°C for 30 min. Chromatographic mobility of marker steroids indicated: (1) progesterone, (2) 5α -pregnane-3,20-dione; (3) 5β -pregnane-3,20-dione; (4) 20α -hydroxypregn-3-one; (5) 20β -hydroxypregn-3-one; (6) 3β -hydroxypregn-20-one; (7) 5β -pregnane-3 α ,20 α -diol (pregnenediol); (8) 5β -pregnane-3 α ,20 β -diol; (9) 5β -pregnane-3 β ,20 β -diol. Note the metabolism of ^3H -progesterone by hyrax blood (b) and the formation of compounds with chromatographic mobilities similar to those of 5α - and 5β -pregnenediol, and of hydroxylated pregnane metabolites with chromatographic properties similar to those of dihydroxypregnane compounds after acetylation.

progesterone (Table 1). In *Heterohyrax* the luteal concentration in pregnancy was $5.8\text{--}24.2 \text{ ng mg}^{-1}$, and one pregnant *Procavia* had 18.6 ng mg^{-1} . Progesterone concentration was low in one animal (J5, Table 1) but this did not seem to be associated with a failing pregnancy. A very low progesterone concentration was found in residual ovarian tissue after the removal of luteal tissue (less than 0.1 ng mg^{-1}) and in the placenta (less than 0.002 ng mg^{-1}).

An unusual feature of the hormone content of the hyrax corpus luteum was the high concentration of immunoreactive unconjugated oestrogens, $35\text{--}1,840 \text{ pg mg}^{-1}$ (Table 1). In contrast, the oestrogen concentration in residual ovarian tissue did not exceed 8 pg mg^{-1} , and in placental tissue it was less than 0.4 pg mg^{-1} .

Plasma concentrations of progesterone in blood taken by cardiac puncture were low in all females ($1.2 \pm 0.3 \text{ ng ml}^{-1}$, $n = 22$). In immature females the concentration was 0.3 and 0.4 ng ml^{-1} , in non-pregnant females, $0.6 \pm 0.2 \text{ ng ml}^{-1}$, and in pregnant females values ranged from less than 0.1 to 4.6 ng ml^{-1} during early, mid and late gestation. In hyrax J16 (Table 1), a mature female housed in laboratory conditions, the concentration in weekly samples varied between 1.5 and 3.8 ng ml^{-1} during approximately 30 d before a fertile mating. In early pregnancy, 4.6 ng ml^{-1} was recorded. The plasma progesterone concentration in males was $0.6 \pm 0.3 \text{ ng ml}^{-1}$ ($n = 8$).

The plasma concentration of unconjugated oestrogens in immature females was less than 10 pg ml^{-1} , in non-pregnant females 138 ± 19 ($n = 19$), and in pregnant females $106 \pm 44 \text{ pg ml}^{-1}$ ($n = 5$). The concentration in males, however, was of the same order, $134 \pm 35 \text{ pg ml}^{-1}$ ($n = 8$), indicating that reactivity was probably nonspecific.

Plasma samples from shot specimens contained a lower concentration of progesterone than those from captive animals obtained under anaesthesia. This loss of activity after sampling seemed to be related to a delay in separating the plasma from shot specimens. ^3H -progesterone incubated with whole blood at 37°C was metabolised (Fig. 1) to compounds corresponding in mobility to ring A saturated compounds less polar than progesterone, and hydroxylated steroids more polar than progesterone (Fig. 2). After incubation at 4°C only 62% of the ^3H -progesterone was recovered and the loss was associated with uptake by the red cells rather than enzymatic metabolism. Blood from shot specimens taken within 5 min of death and extracted immediately with diethyl ether gave progesterone values of 1.1 and 1.8 ng ml^{-1} (corrected for procedural losses) in two early-mid-pregnant females. These values were similar to those obtained in plasma from captive animals, and indicated that the amount of progesterone sequestered in erythrocytes was not large.

The amount of ^3H -progesterone recovered from hyrax blood after a 120-min incubation at 37°C was similar in non-pregnant and pregnant hyraxes (range $0.8\text{--}13.3\%$). The metabolism and uptake was attributable to erythrocytes since incubations with

laboratory at 4°C . Blood was taken from captive animals by cardiac puncture under chloroform anaesthesia. Plasma was separated by centrifugation within 15 min of collection from these animals, but at least 3 h elapsed before plasma could be obtained from shot specimens. Plasma and tissue samples were either extracted immediately or stored at -15°C . Progesterone and total unconjugated oestrogens were determined by radioimmunoassay (method A in ref. 8 and ref. 9). Tissue progesterone concentrations were corrected for procedural losses assessed by the recovery of a tracer amount of ^3H -progesterone added to each tissue. Details of these procedures will be published later.

The corpus luteum during pregnancy varied in wet weight between 13 and 18 mg and contained a significant amount of

Table 1 Concentration of progesterone and total unconjugated oestrogens in the corpus luteum of *Heterohyrax brucei*

Animal no.	Condition of animal	No. of foetuses and distribution	Weight of foetus (g); C-R length (mm)	Mean wet weight (mg)*	Progesterone concentration (ng mg ⁻¹)	Corpus luteum Progesterone content (ng)	Oestrogen concentration (pg mg ⁻¹)	Oestrogen content (ng)
J12	Non-pregnant	—	—	16 (2)	26.1	417.6	682	10.9
J16	Early pregnancy	2 (1L, 1R)	2.65†	18 (3)	14.8	266.4	35	0.6
J13‡	Early pregnancy	2 (1L, 1R)	—	13.5 (2)	18.6	251.1	139	1.9
D6	Early-mid-pregnancy	2 (1L, 1R)	10; 75	16 (2)	23.3	372.8	650	10.4
D4	Early-mid-pregnancy	2 (1L, 1R)	10; 75	14 (2)	24.2	338.8	1,840	25.8
J2	Mid-pregnancy	2 (1L, 1R)	25; 98	14 (2)	15.9	222.6	820	11.5
J5	Mid-pregnancy	2 (1L, 1R)	35; 110	13 (2)	5.8	156.6	620	16.7
J4	Late pregnancy	1 (1L)	70; 140	14 (2)	21.6	302.4	1,600	22.4

*Numbers in parentheses indicate number of corpora lutea.

†Weight of conceptus

‡*Procavia* sp.

an equivalent volume of red cells resulted in recoveries of only 11.0 and 12.0% of added ³H-progesterone. The rate of disappearance of ³H-progesterone was reduced if the erythrocytes had been lysed with distilled water; the average amount of labelled progesterone recovered being 41.1%. The enzymatic activity of erythrocytes with respect to ³H-progesterone was abolished by the addition of NaF.

The progesterone requirements of pregnancy may be met in various ways in different mammals¹⁰. In rats, sheep and women there is an increased production of progesterone; in guinea pigs there is a marked decrease in its rate of clearance and only a modest rise in production. The hyrax does not seem to fit either of these categories, since no evidence has been found of a high concentration of plasma progesterone in gestation, or of a pregnancy-specific plasma protein with a high affinity for progesterone.

The ability of erythrocytes to metabolise steroid hormones, including progesterone, has been reported in several species including man¹¹⁻¹⁵, but only in the foetal lamb, calf and kid has a rapid conversion of progesterone to 20 α -dihydroprogesterone been found^{14,15}. Although the two studies were not directly comparable, the rate of erythrocyte metabolism of progesterone *in vitro* seemed to be less in the hyrax than in the foetal lamb. In both species, however, cell lysis reduced enzymatic activity. In the hyrax, NaF, an inhibitor of anaerobic glycolysis, blocked progesterone metabolism which further supports the suggestion that steroid dehydrogenases in red cells utilise NADH generated by the glycolytic pathway¹².

A low plasma progesterone concentration during pregnancy has also been reported in the tammar wallaby¹⁶, but these marsupials have a gestation period of only 28 d. Maintenance of pregnancy by low plasma progesterone levels in the hyrax may be facilitated by a particularly sensitive receptor mechanism in the target tissues, with blood metabolism contributing to the protection of maternal and foetal tissues from any adverse effects of high hormone concentrations. There remains the controversial but interesting possibility that support of gestation in these animals depends on a metabolite, perhaps associated with erythrocyte metabolism of progesterone, rather than on progesterone itself. This possibility recalls work on the elephant which has cast doubt on the role of progesterone as the hormone of pregnancy⁷. Although the progesterone concentration in the corpus luteum of the hyrax contrasts with the low level reported in the elephant, the plasma levels in both animals are low during gestation. In neither animal does the endocrinology of pregnancy conform to patterns described in other mammals.

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Pressure reversal of narcosis produced by anaesthetics, narcotics and tranquillisers

THE first demonstration of pressure reversal of anaesthesia in amphibians was provided by the tadpole experiments of Johnson and Flagler¹ with ethanol and ethyl carbamate. Since then the phenomenon has been studied using general anaesthetics in newts, mice and marine organisms^{2,3}, but few of these agents have been studied in detail and the results in some cases, for example, for barbiturates^{4,5}, are conflicting. Another aspect of the interaction of pressure and anaesthesia is the amelioration of the high pressure nervous syndrome by nitrous oxide, hydrogen or nitrogen⁶⁻⁸. It is not, however, known if other agents can also give 'pressure protection', and tadpoles provide a relatively easy model for a preliminary screening of potentially suitable drugs. We have studied both these aspects of the interaction between pressure and narcosis using intravenous, local and inhalational general anaesthetics and tranquillisers. We show that pressure reversal occurred with all our agents pro-

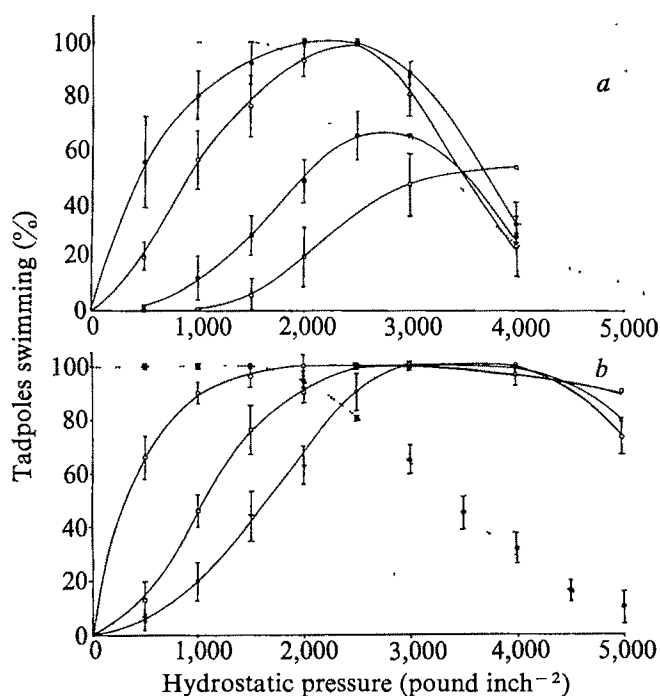


Fig. 1 *a*, Effect of pressure on tadpoles swimming in water equilibrated with different concentrations of halothane. ●, 0.005 atm; ○, 0.0075 atm; ■, 0.01 atm; □, 0.015 atm. Error bars indicate ± 1 s.e.m. calculated from five groups of five tadpoles. Dotted line shows effect of pressure alone. The tadpoles (*Rana temporaria*) were immersed in an aqueous solution of the agent at a known partial pressure and equilibrated for up to 30 min before pressurising to avoid any transitory effects associated with uptake and distribution of the agent. The chamber (400 ml capacity) was completely filled with solution which provided sufficient oxygen throughout the experimental period (up to 15 min). Pressure was applied using an oil-filled pump connected by way of a side chamber to prevent contamination. In almost all cases the observed effects were completely reversible, although recovery could take up to 18 h. *b*, Effect of pressure on tadpoles equilibrated with althesin—an intravenous steroid anaesthetic consisting of alphaxalone (3 parts by weight), alphadalone acetate (1 part by weight) in cremophor E. L. plus saline; (Cremophor E. L. was tested alone to ensure its inactivity). Concentrations were expressed as total steroid in $\mu\text{g ml}^{-1}$. ●, No anaesthetic present (dotted line); ○, 3.0 $\mu\text{g ml}^{-1}$; □, 4.2 $\mu\text{g ml}^{-1}$; ▽, 6.0 $\mu\text{g ml}^{-1}$. Error bars indicate ± 1 s.e.m. calculated from five groups of five tadpoles.

ducing narcosis in tadpoles, whereas pressure protection does not seem to be as universal.

Tadpole swimming activities in the presence of increased hydrostatic pressure with or without halothane or althesin are shown in Fig. 1*a* and *b*. It was only possible to quantify the response in terms of the presence or absence of movement (which was either spontaneous or initiated by tapping the side of the chamber). With pressure alone there was a progressive (but reversible) decrease in swimming activity—termed ‘pressure paralysis’—when the tadpoles became generally flaccid. In contrast to the inactivity produced by narcosis, however, pressure paralysis was normally accompanied by a pronounced bending of the tail. Other pressure effects included hyperactivity at 1,000–1,500 pound inch⁻² and tremors at 3,000–4,000 pound inch⁻².

The tadpoles were initially inactive in the presence of narcotics, but as the hydrostatic pressure increased they resumed swimming. The variation of narcotic reversal pressure with narcotic dose gives some indication of the differing degrees of pressure reversal. We found that the magnitude of the effect was too large to be attributed solely to the expected change of solubilities with pressure⁹. Decompression at the point of narcotic reversal produced an immediate return to narcosis which was again reversed by recompression. For both agents the effect of increasing the pressure was biphasic, although with althesin pressure paralysis only occurred above 5,000 pound

inch⁻². At the two highest halothane partial pressures the animals never fully recovered from narcosis before the onset of pressure paralysis.

Other agents were investigated using groups of five tadpoles and the results, expressed in terms of the onset of narcotic reversal and pressure paralysis at different doses of agents, are shown in Tables 1 and 2. The agents listed in Table 2 are not normally considered to be satisfactory general anaesthetics but, when the doses are sufficient to cause narcosis in tadpoles, pressure reversal can still occur. It has been postulated that benzocaine and procaine produce their local anaesthetic effects by membrane expansion, whereas agents such as lignocaine act at specific charged sites¹⁰. All three local anaesthetics however, had their narcotic effects reversed by pressure, which is consistent with the hypothesis that there is expansion of the critical site for narcosis independent of the agent.

Table 1 Narcotic reversal and pressure paralysis in tadpoles influenced by clinical anaesthetics

Agent	'Dose'	Complete narcotic reversal (atm)	Complete pressure paralysis (atm)
Methoxyflurane	0.001 atm	51	272
	0.002 atm	204	340
Chloroform	0.0025 atm	34	306
	0.005 atm	170*	272
Halothane	0.005 atm	102	306
	0.0075 atm	170	306
	0.01 atm	187*	306
	0.015 atm	238*	340†
Trichloroethylene	0.005 atm	68	306
	0.0077 atm	102*	272
Enflurane	0.008 atm	102	374
	0.015 atm	170	374
Diethyl ether	0.01 atm	68	408
	0.02 atm	136	> 408
Cyclopropane	0.1 atm	102	306
	0.15 atm	136	306
Nitrous oxide	0.8 atm	51	408
	3 $\mu\text{g ml}^{-1}$	136	> 408
Althesin	4.2 $\mu\text{g ml}^{-1}$	170	> 408
	6 $\mu\text{g ml}^{-1}$	204	> 408
Methohexitone	0.1 mM	102	272
	0.13 mM	102	306
	0.17 mM	238	340
	0.89 mM	102	> 272
† Propanidid	1.9 mM	136	340
‡ Thiopentone			

* Pressure at which maximum response was obtained when complete pressure reversal did not occur.

† These animals did not recover at the end of the experiment.

‡ Lethal – narcotising dose ratio close to unity.

Our results with ethanol are in agreement with those of Johnson and Flagler¹, who used different species of tadpoles and larvae. A high dose of morphine produced tadpole narcosis but there was no effect with two other narcotic analgesics, fentanyl citrate and pentazocine. The pressure reversal of the narcosis produced by the different tranquillisers emphasises that this phenomenon is not associated with specific agents or groups of drugs.

When studying the amelioration of the high pressure nervous syndrome we noted that tremors never occurred in the presence of narcotics, but since this effect was difficult to quantitate, we have not included it in our characterisation of ‘pressure protection’. With pressure alone, 100% activity is lost above 1,470 pound inch⁻², which is equivalent to 100 atm. Halothane seemed to give no significant protection against the paralyzing effects of high pressure (Fig. 1*a*); the animals exposed to 0.015 atm halothane died at 330 atm. In Tables 1 and 2 any figure for complete narcotic reversal above 100 atm indicates some degree of pressure protection. Several agents protected the tadpoles to some extent against the effects of very high pressure; these have a pressure paralysis value of > 300 atm. Althesin was the most successful agent that we used and is shown in Fig. 1*b*. This protective effect of althesin does not

Table 2 Narcotic reversal and pressure paralysis in tadpoles influenced by unconventional anaesthetics

Agent	'Dose'	Complete narcotic reversal (atm)	Complete pressure paralysis (atm)
†Lignocaine (pH 6.95)	1 mM	68	272
†Procaine (pH 7.20)	0.88 mM	34	238
Benzocaine (pH 7.23)	0.24 mM	34	306
Ethanol	0.43 M	68	272
	1.37 M	136	272
†Morphine	0.55 mM	34	204
†Chlorpromazine	0.16 mM	102	238*
Diazepam	0.09 mM	102	272
	0.18 mM	238	272*
†Droperidol	0.33 mM	68	272
†Promazine	0.31 mM	68	272*

*These animals did not recover at the end of the experiment.

†Lethal - narcotising dose ratio close to unity.

seem to apply to all steroids; for example, hydrocortisone, a non-anaesthetic steroid, gave no pressure protection.

Our results demonstrate that the phenomenon of pressure reversal applies to narcosis in tadpoles regardless of the agent used. The compounds included a wide range of drugs with a very large variation in both anaesthetic potency and molecular size. These results may be relevant to the analogous situation in mammals since the ratio of tadpole narcotic concentration to the AD_{95} for surgical anaesthesia in man¹¹ is 2.75 ± 0.62 (s.d.), which has a coefficient of variation within that predicted by the accuracy of both measurements. Pressure reversal occurring with the steroids in althesin is particularly interesting because their anaesthetic activity in mammals seems to be related to their stereochemistry, and some workers have assumed that their mode of action is fundamentally different from the inhalational agents¹². Our data suggest that, although there may be some underlying minor differences in the physico-chemical basis of the expansion at the site of action, there are no intrinsic differences in the molecular mechanism of narcotic action of the inhalational and intravenous agents we studied.

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A leukaemic cell mutant with a thermolabile alanyl-transfer RNA synthetase

It may be of great help in the elucidation of regulatory systems in mammalian cells if we could isolate temperature-conditional mutants and identify their mutated functions. I report here that

a temperature-sensitive mutant of L5178Y murine leukaemic cells has a thermolabile L-alanyl-tRNA synthetase.

The *ts3* cells have been shown to exhibit a mutant character when grown in Fischer's medium but not in Ham's F12 medium, that is, cells grow at the non-permissive temperature (39 °C). L-alanine (10^{-4} M), hypoxanthine (3×10^{-5} M), and sodium pyruvate (10^{-3} M) are components of Ham's F12 medium but not Fischer's, and are responsible for growth of the mutant cells at the elevated temperature¹. Each of these substances was examined at various concentrations for growth stimulation.

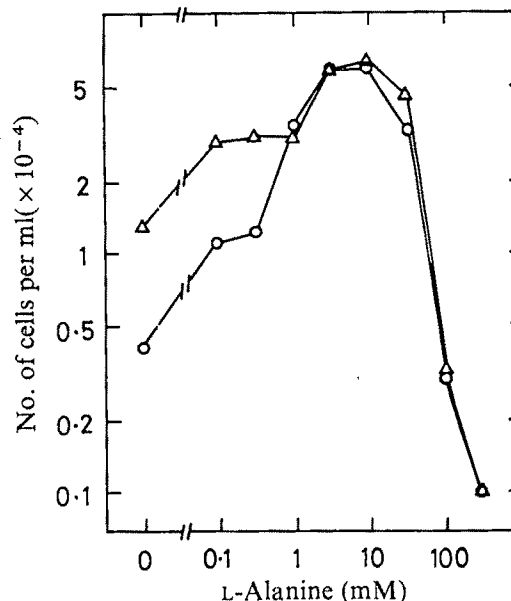


Fig. 1 Effect of L-alanine on growth of *ts3* cells at 39 °C. 1×10^4 *ts3* cells ml^{-1} were inoculated in dialysed calf serum-containing Fischer's medium supplemented with various concentrations of L-alanine with (Δ) or without (○) hypoxanthine (3×10^{-5} M) and sodium pyruvate (10^{-3} M). After incubation at 39 °C for 3 d the cell numbers were scored.

For hypoxanthine and pyruvate the optimal concentrations were the same as those contained in Ham's F12 medium. Varying concentrations of alanine, however, exerted great influence on cell growth. As shown in Fig. 1, at 0.1 and 0.3 mM L-alanine, both hypoxanthine and pyruvate are required for *ts3* cells to grow at 39 °C. Above concentrations of 1 mM L-alanine, hypoxanthine and pyruvate are dispensable and alanine alone can support growth. No other L-amino acids, nor D-alanine, nor β-alanine could be substituted for L-alanine. Cell growth was inhibited at concentrations higher than 30 mM alanine. In the lower range of alanine concentrations pyruvate may serve as a precursor of alanine and be converted to the amino acid in the cell. Hypoxanthine has been shown to have a growth-promoting effect for L5178Y cells (T. Shiomu, personal communication).

Since *ts3* cells were shown to require alanine for growth at 39 °C, I examined the possibility that alanine biosynthesis was impaired at a restrictive temperature in the mutant. The main pathway of alanine synthesis is a conversion of pyruvate and L-glutamate to L-alanine and α-ketoglutarate and the reaction is carried out by alanine aminotransferase (EC 2.6.1.2). The enzyme activity was determined after exposing cell extracts to a high temperature². The initial specific activities expressed as μmol pyruvate formed per min per mg protein were 14.0 and 15.2 and the residual activities after 60 min at 40 °C were 11.6 and 12.8 for wild-type and *ts3* cells, respectively. This shows that the enzyme activity of *ts3* cells was as stable as that of parental wild-type cells at the elevated temperature, the residual activities being more than 80% for both wild-type and mutant cells. This result indicates that the main biosynthetic pathway of alanine is not altered in *ts3* cells.

The rates of incorporation of radioactive amino acids and

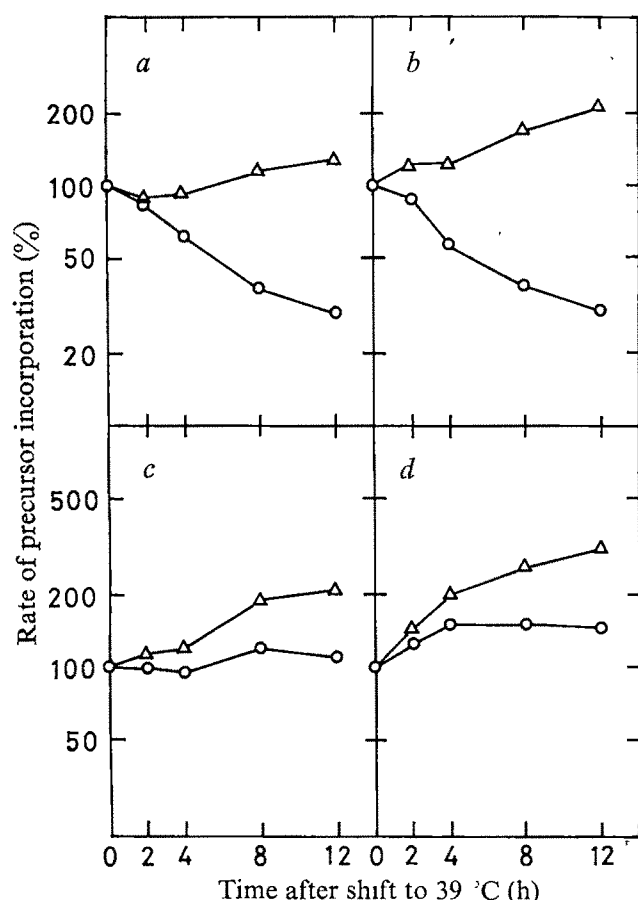


Fig. 2 Rates of incorporation of radioactive amino acids and nucleosides into acid-precipitable material after shift from 33 to 39 °C for *ts3* (○) and wild-type (Δ) cells. At the indicated times, cells were labelled for 30 min with either: a, ^{14}C -alanine (159 Ci mol^{-1} , $0.8 \mu\text{Ci ml}^{-1}$); b, ^{14}C -thymidine (49.5 Ci mol^{-1} , $0.1 \mu\text{Ci ml}^{-1}$); c, ^{14}C -valine (150 Ci mol^{-1} , $0.2 \mu\text{Ci ml}^{-1}$) or d, ^{14}C -uridine (59 Ci mol^{-1} , $0.01 \mu\text{Ci ml}^{-1}$). Ice-cold 5% TCA-insoluble material was collected on Whatman GF/F glass fibre filters, washed with TCA and ethanol, and counted in a toluene-based liquid scintillation counting fluid. Radioactive materials were purchased from Daiichi Pure Chemical Co., Ltd, Tokyo, and The Radiochemical Centre, Amersham.

nucleosides into acid-insoluble material were measured after shift from 33 to 39 °C for mutant and wild-type cells. As shown in Fig. 2, a steady reduction with time was observed in the rates of incorporation of alanine and thymidine in *ts3* cells, the level reaching 30% after 12 h (Fig. 2a and b), whereas the incorporation of valine and uridine did not change much during a 12-h period in mutant cells (Fig. 2c and d). It has been shown with *ts3* cells that leucine incorporation is unaffected for the first 24 h at the elevated temperature¹. Wild-type cells, by contrast, incorporated an increasing amount of all these precursors into macromolecules as the incubation time was prolonged (Fig. 2). Although the decline of thymidine uptake was observed in parallel with that of alanine uptake in mutant cells, this is not necessarily an indication that the DNA replicating machinery is also thermolabile. Since simultaneous protein synthesis is a requisite for DNA replication to initiate and continue in eukaryotic cells³, we could argue that the decreased synthesis of DNA results from the reduced incorporation of alanine provided that alanine is essential to the synthesis of the proteins required for DNA replication. The result that the incorporation of alanine, but not of leucine or valine, was affected at the restrictive temperature indicates that an alanine-specific process is modified in the mutant.

In bacteria many mutants auxotrophic for an amino acid contain an altered aminoacyl-tRNA synthetase⁴. Mutants with a temperature-sensitive alanyl-tRNA synthetase incorporate alanine at a decreasing rate but continue to incorporate leucine

Table 1 Thermal inactivation of aminoacyl-tRNA synthetases of wild-type and mutant *ts3* cells

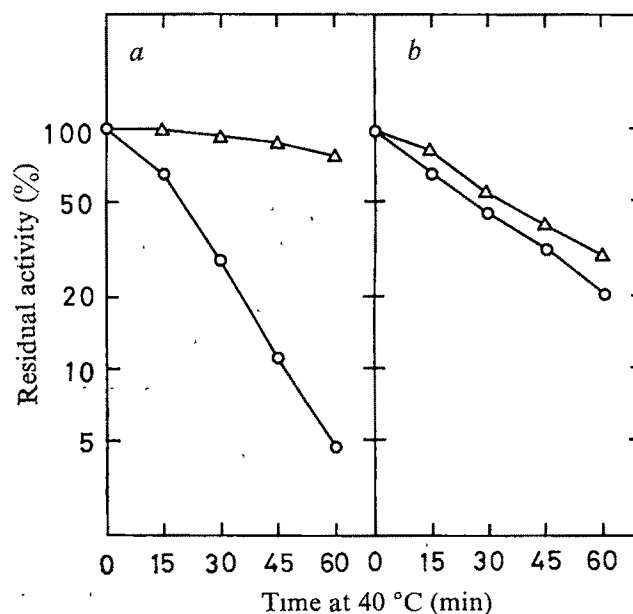
^{14}C -amino acid	Residual activity (%) after 60 min incubation at 40 °C	
	Wild type	<i>ts3</i>
Alanine	77.8	4.7
Arginine	12.2	11.7
Asparagine	65.4	67.1
Aspartic acid	53.2	79.1
Cysteine	38.5	28.6
Glutamic acid	12.4	13.7
Glutamine	16.0	12.8
Glycine	29.8	25.6
Histidine	15.3	14.4
Isoleucine	40.5	59.3
Leucine	28.9	19.9
Lysine	37.4	39.8
Methionine	75.3	87.3
Phenylalanine	59.9	60.6
Proline	8.4	8.1
Serine	62.3	54.7
Threonine	41.1	30.7
Tryptophan	78.7	94.5
Tyrosine	37.4	25.6
Valine	51.4	54.5

pH 5.2 fractions were prepared from wild-type and mutant cells and exposed to 40 °C for 0 and 60 min. Aminoacyl-tRNA synthetase activities were assayed for each amino acid as described in Fig. 3.

at a constant linear rate after exposure to the high temperature⁵. These findings suggest the possible involvement of alanine-activating enzyme in *ts3* cells.

The thermal inactivation of L-alanyl-tRNA synthetase (EC 6.1.1.7) was examined. The "pH-5.2 fraction" was prepared⁶ from mutant and wild-type cell extracts and exposed to 40 °C for various lengths of time. The residual activity was measured at 30 °C for alanine and leucine (Fig. 3). The alanyl-tRNA synthetase from *ts3* cells was far more rapidly inactivated than that from wild-type cells at 40 °C (Fig. 3a), whereas the leucyl-tRNA synthetases from mutant and wild-type cells were

Fig. 3 Thermal inactivation of alanyl- and leucyl-tRNA synthetases of wild-type (Δ) and *ts3* (○) cells. From cell homogenates, pH 5.2 fractions were prepared⁶ and used for assaying alanyl- (a) and leucyl-tRNA synthetase (b) activities after incubation at 40 °C for indicated periods of time. Each 0.1 ml reaction mixture contains: 100 mM Tris-HCl (pH 7.5), 10 mM KCl, 4 mM magnesium acetate, 5 mM ATP, 2 mM dithiothreitol, 10 μM each of 20 L-amino acids, 2 mg ml⁻¹ rat liver tRNA (Grand Island Biological Co., Grand Island, New York), 15 μM ^{14}C -alanine (159 Ci mol^{-1}) or 15 μM ^{14}C -leucine (280 Ci mol^{-1}), and cell extract. Reactions were carried out at 30 °C for 15 min and terminated by the addition of ice-cold 10% TCA. Precipitates were collected on Whatman GF/F glass fibre filters, washed and counted.



inactivated at approximately the same rate (Fig. 3b). The residual activities of alanyl-tRNA synthetases after 60 min incubation at 40 °C were 5 and 78% and those of leucyl-tRNA synthetases were 20 and 29% for mutant and wild-type cell extracts, respectively. These results demonstrate that alanyl-tRNA synthetase activity of *ts3* cells is far more thermolabile than that of wild-type cells.

To determine whether the altered function is alanine specific, the heat stability of the other aminoacyl-tRNA synthetases was studied. As shown in Table 1, there is little difference in the residual activities between wild-type and mutant cell extracts for all amino acids except alanine. The result clearly shows that the elevated temperature inactivates each aminoacyl-tRNA synthetase from wild-type and mutant cells to approximately the same level with the exception of alanyl-tRNA synthetase.

The extreme lability of the alanyl-tRNA synthetase of the mutant in *in vitro* conditions will explain the temperature-sensitive growth character of *ts3* cells. Further purification of the enzyme will reveal whether the affinity for L-alanine or the recognition site for tRNA is altered as shown in bacterial mutants^{7,8}. A Chinese hamster ovary cell mutant with a temperature-sensitive leucyl-tRNA synthetase has been reported⁹. There is a striking difference between the leucine mutant and *ts3* cells; valine incorporation is affected in the former but not in the latter in the non-permissive condition. The continued valine incorporation in *ts3* cells may represent synthesis of proteins that are low in, or lack, alanine or of abnormal polypeptide derivatives⁵. Uridine incorporation into RNA does not change much with time in spite of the depletion in alanyl- or leucyl-tRNA, which may be accounted for by assuming that either the pool of uncharged tRNA has not reached a level sufficiently high to cause a general shut-off in transcription or the stringent control does not operate in high eukaryotes. A decline in DNA synthesis is observed in both mutants and explained as a secondary consequence of reduced synthesis of initiator proteins of DNA replication. These points are, however, to be substantiated.

Non-conditional alanine-requiring mutants have been described¹⁰ which may carry a similar mutation.

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three of these head proteins are found intact in the phage; the products of genes *E* and *D*, pE and pD, form the outer shell of the capsid⁶, and pF_{II} is thought to form part of the head-tail connector⁷. The other two gene products pB and pC undergo extensive alterations during head assembly. pC is fused to a minor fraction of pE and is cleaved to form two polypeptides, X1 and X2, which differ only in the length of the pC fragment present⁸. There are between three and nine molecules of each of these proteins in the phage head⁶. The product of gene *B*, pB, is also cleaved during head assembly to form pB* (refs 4–6). There are between 10 and 14 molecules of pB* in the phage^{3,6}. Since an icosahedron has 12 corners, it has been suggested that 12 pB* molecules are structural components of the corners of the viral capsid^{9,11}.

We suggest, however, that the pB* molecules are not distributed in the corners of the icosahedral viral shell but that, together with X1 and X2, they form the collar structure which connects the head and the tail. We have based these conclusions on the following observations. Petit λ particles (pλ), which appear in electron micrographs to be less angular in outline, and about three-quarters the diameter of λ heads (Fig. 1)¹², are normal precursors of mature λ heads^{11,12,24,25}. Particles similar in appearance, although not able to act as head precursors, can be obtained in equivalent amounts in the absence of pB and pC (refs 11, 14 and 15). According to the general principles regulating the construction of isometric viral shells, elaborated by Caspar and Klug, pλ particles must have 12 corners¹⁶. Therefore the presence of structures like pλ in B[−] and C[−] lysates indicates that pB and pC are not essential for the formation of corners. The less angular outline of all pλ relative to λ can be explained simply in terms of the grouping the major structural proteins pE and pD on the surfaces of pλ and the mature head capsids as follows. In pλ, 420 molecules of pE are thought to be grouped as hexamers and pentamers to give 72 morphological capsomers^{17,24}. During maturation of the proheads, however, the pE molecules are regrouped as trimers and 420 molecules of pD are added to the shell, grouped as hexamers and pentamers, yielding a total of 212 capsomers one-half the size of the pE-containing pλ capsomers^{17,18,24}. The decrease in size and the increase in the total number of capsomers in λ heads could explain the observation that mature phage heads are more angular than pλ.

The collar structure observed in electron micrographs of λ heads (Fig. 1) is presumably derived from the head, since tails acquire collars only after their attachment to heads^{11,19–22}. Furthermore, it is likely that the collar is a preformed head structure and not simply a rearrangement of capsid proteins in response to the addition of a tail. Harrison and Bode (cited in ref. 21) have argued that such major structural alterations are unlikely in view of the low activation energy of head-tail joining reaction *in vitro* (8.5 kcalorie mol^{−1}).

A rough estimate of the size of the collar structure based on measurements from the electron micrograph in Fig. 1b gives a molecular weight of 1 × 10⁶. Thus, the five or six molecules of pF_{II} (molecular weight 12,000) present per phage⁷ could not make up the bulk of the material seen in electron micrographs (Fig. 1b). Furthermore a collar-like structure can be seen in phage heads and in pλ in F_{II}[−] mutant lysates (Fig. 1g). Clearly this structure must contain other head proteins in addition to the five to six molecules of pF_{II}.

The presence or absence of a collar-like structure in empty heads and in proheads can be correlated with the presence or absence of pB*, X1 and X2 based on examination of the structures found in lysates of phage mutants. Empty heads found in lysates of a *Vam* tail defective mutant (Fig. 1c) have a structure which appears under the electron microscope to be very similar to the tail-collar

Model for arrangement of minor structural proteins in head of bacteriophage λ

THE morphogenesis and maturation of the head of bacteriophage λ requires the function of 10 phage genes^{1–5}, with the products of five of these genes, *E*, *D*, *F*_{II}, *B* and *C*, present as structural components of the head^{4,6,7}. The first

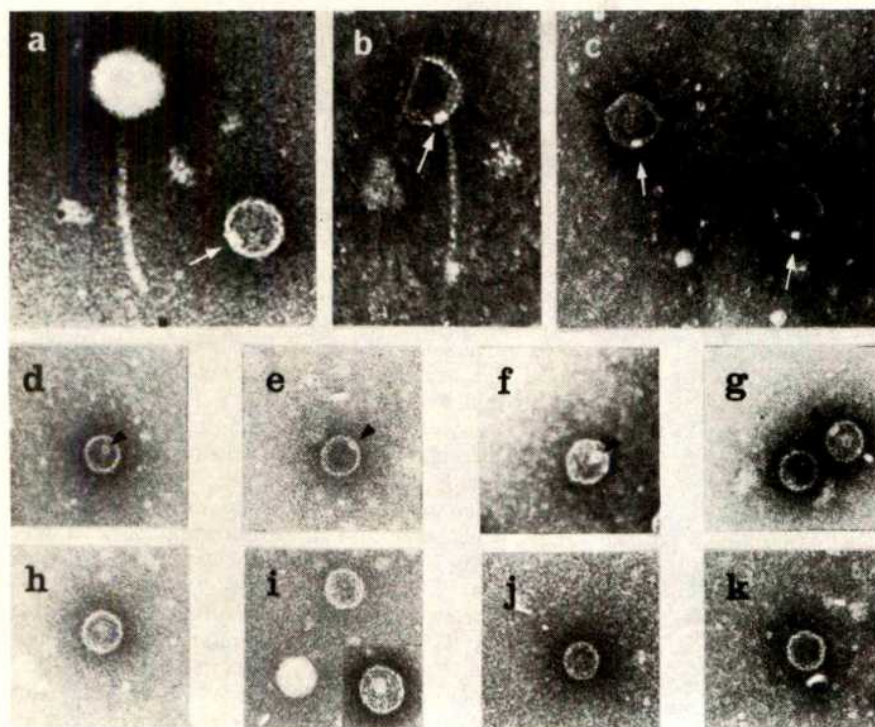


Fig. 1 Presence of tail collar in phage and pλ. Typical structures found in lysates with collar structures indicated by arrows. *a*, Lysate obtained after induction of 594(λ Cl₈₅₇Sam₇) showing a complete DNA-filled phage particle and a pλ with a collar. *b*, Ghosted phage showing collar joining tail to head. *c*, Empty heads from a lysate of 594(λ Cl₈₅₇Vam₇₅₀Sam₇). *d-g*, pλ with collar from lysates of induced cultures of: *d*, 594(λ Cl₈₅₇Aam₃₂₇Sam₇); *e*, 594(λ Cl₈₅₇Dam₁₅Sam₇); *f*, 594(λ Cl₈₅₇F₁₁am₇₈₅); *g*, 594(λ Cl₈₅₇F₁₁am₄₂₃Sam₇). A collar structure was clearly visible in about 30–50% of the pλ in the above lysates. *h-k*, pλ showing the absence of the collar, from lysates of induced cultures of: *h*, 594(λ Cl₈₅₇Bam₁₀Sam₇); *i*, 594(λ Cl₈₅₇Cam₄₂Sam₇); *j*, 594(λ Cl₈₅₇Bam₁₁Cam₄₂Sam₇); and *k*, 594(λ Cl₈₅₇Nu3am₈₈Sam₇). For a more complete description of the strains used, see refs 14 and 23. The preparation of the lysates and the procedures used for electron microscopy were described previously²³. *a-b*, $\times 160,000$; *c*, $\times 120,000$; *d-k*, $\times 100,000$.

found in disrupted phage heads (Fig. 1*b*). This structure is also found in pλ from wild-type lysates and from lysates of A^- , D^- , F_I^- and F_{II}^- phage-infected cells (Fig. 1*a* and *d-g*). Polyacrylamide gel electrophoretic analysis has indicated that all of these types of pλ particles contain pE, pB*, X1 and X2 (refs 9, 11 and 23). The collar-like structure is not found in pλ which do not contain pB*, X1 or X2, that is, pλ from lysates of B^- , C^- , B^-C^- or $Nu3^-$ phage-infected cells (Fig. 1*h-k*). Lysates of λC^- -infected cells contain three morphologically distinct pλ (Fig. 1*i*). Some appear completely empty, some appear completely full, and some have a small inclusion which is similar to the collar structure seen in wild-type proheads (Fig. 1*a*). In C^- pλ, however, this inclusion is larger and more diffuse than wild-type pλ collars, and may be unprocessed pB or pNu3 which has been found in these pλ (refs 9, 11 and 23). Since pE forms the outer shell of the pλ particle and is found in all types of pλ, regardless of whether they contain the collar-like

structure^{9,11,23}, it seems likely that the structure seen in Fig. 1*a-g* is made up of pB*, X1 and X2, rather than pE.

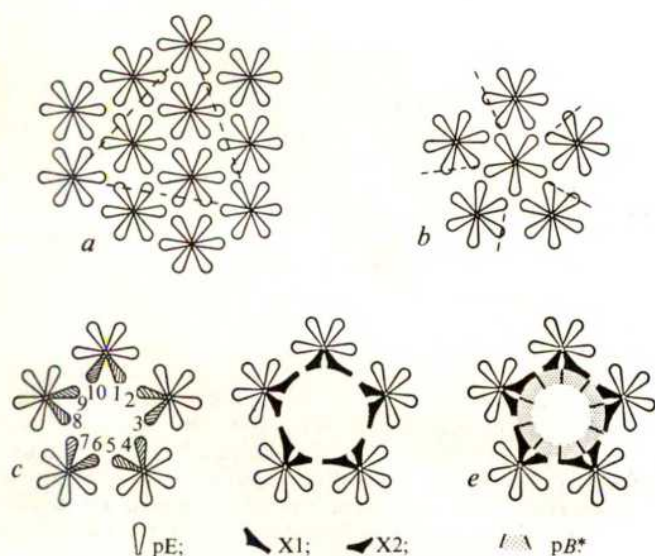
Previous data²³ suggest that pB and pC interact, and that the cleavage of pB requires the previous cleavage of pC. Thus it seems more likely that these proteins share a common function in the prohead, rather than being dispersed over the capsid.

These observations suggest that pB*, X1 and X2 form part of the head-tail connector. Figure 2 shows a model to explain how these molecules might be attached to a single corner of the head. The model proposes that the cleavage and fusion of pC and pE to form X1 and X2 takes place at only one of the 12 corners of the icosahedral shell.

Figure 2*a* shows a flat lattice structure of pE molecules grouped as hexamers as has been suggested for the molecular arrangement of the pλ capsid^{17,24}. Superimposed on this lattice is the outline of one face of the icosahedral structure. A corner can be generated from this planar hexagonal net with minimum distortion of the bond angles by cutting along one of the sides of the icosahedral face (Fig. 2*a*, broken lines) and folding the net into a cone to convert the corner capsomer to a pentamer¹⁶. The corner thus generated (Fig. 2*b*) consists of a pentamer of pE surrounded by five pE hexamers. The five faces of the icosahedron forming this corner are indicated by broken lines in Fig. 2*b*. If the pE pentamer is removed from this corner the resulting hole is surrounded by 10 molecules of pE (Fig. 2*c*, cross hatching). These 10 molecules of pE are not in identical molecular environments, but rather form two groups. One group, the even-numbered molecules in the figure, is oriented such that the left side of each molecule is facing the centre of the corner. The odd-numbered molecules have their right side facing the centre of the corner. We propose that 10 pC molecules are fused to these 10 pE molecules and are subsequently cleaved to form five molecules of X1 and five of X2 (Fig. 2*d*). The different molecular environments of the pE molecules could explain the presence of two sets of molecular species, X1 and X2, which have the same amino acid sequence but differ only slightly in length⁸. Ten molecules of pB* would then be located in contact with the ten X molecules to form the collar (Fig. 2*e*) seen in Fig. 1*d-g*.

Our previous results²³ suggest that pNu3 is involved in the assembly and functional cleavage of pB and pC. pNu3 has not been included in the model (Fig. 2) primarily because

Fig. 2 Model for the arrangement of the minor structural proteins of the head, pB*, X1 and X2, in the collar structure. See text for details.



it has a transient role in assembly and is not a structural component of the mature virion. It is possible that pNu3 provides a substructure to which pB and pC bind to form a precollar structure. This structure could then function as a prime corner which may serve as an initiation point directing the addition of pE to give a complete pλ. The processing of pB and pC would then produce the collar. This model would then provide a mechanism which would prevent pλ from developing more than one collar which might render them inactive in further head assembly. It might also provide an explanation for the formation of monsters in B⁻, C⁻ and Nu3⁻ lysates, which could be produced by the non-directed assembly of pE occurring in the absence of the pre-collar structure. B⁻, C⁻ and Nu3⁻ lysates contain large numbers of pλ; a prime corner is therefore not essential for pλ formation although it may be required for functional pλ assembly as the pλ isolated from these lysates do not act as preheads in the *in vitro* DNA packaging assay¹¹. There are insufficient data available to determine whether collars nucleate the assembly of pE or whether they are added to pre-existing pλ.

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¹⁵N nuclear magnetic resonance of living cells

DIRECT observation of nuclear magnetic resonance (NMR) signals in living tissues, cells, or in their particulate components is an appealing aspect of NMR spectroscopy as it further extends its applicability from the chemical to the biological domain. Of the various nuclei available for NMR studies, ¹H (refs 1-3), ¹³C (refs 2 and 4) and ³¹P (ref. 5) have already been observed in complex biological systems. The potential of the technique for the study of dynamic metabolic processes, or, eventually, aspects of cellular physiological changes, has been demonstrated^{6,7}. No other spectroscopy affords the resolution and direct interpretability in terms of chemical functional groups that NMR provides. One drawback is its relative insensitivity, especially when the representative nuclides of biological interest occur in low natural abundance, for example, ¹³C, ¹⁵N. In the case of ¹³C, isotopic enrichment from suitably labelled precursors has been shown to provide most valuable

metabolic data in whole cell investigations⁸. There seem however, to be no studies of this type using nitrogen NMR. The relevance of nitrogen as a fundamental element of the constitutive building blocks of lipids, nucleic acids, proteins, and even some sugars, suggests that its direct observability in cells should provide an important probe to monitor the dynamics and control of its *in vivo* fixation and metabolism. We report here some results showing that ¹⁵N-NMR signals can be directly observed in whole, living cells.

The fungus *Ustilago spheerogena* was grown at 30 °C in the low-iron media⁹ in which 95% ¹⁵N-ammonium acetate (Merck, Sharp and Dohme, Canada) was used as the sole nitrogen source⁹. Iron starvation, resulting in slow cell growth, was required to stimulate the production of ¹⁵N-enriched ferri-chrome peptides which are the subject of additional ¹⁵N-NMR investigations. The cells were collected and concentrated by centrifugation on day 17 after inoculation. Such cells are perfectly healthy as they can be routinely used as inoculum to start growth of new cultures. From 3.3 l culture medium the

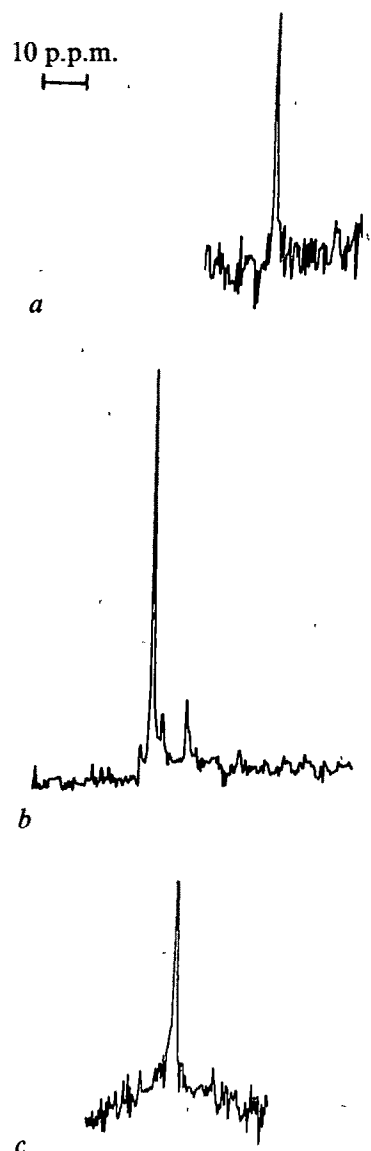


Fig. 1 ¹H-noise decoupled Fourier transform ¹⁵N-NMR spectra at 10.1 MHz of an ¹⁵N-enriched culture of *U. spheerogena*. a, Culture medium free of cells; b, freshly collected cell slurry; c, cell slurry aged for 9 d at 3 °C, and washed with distilled water before the NMR measurement. Spectra were taken at 32 °C on a Varian XL100-15 spectrometer. The chemical shift scale (p.p.m.) is referred to the external ¹⁵NO₃⁻ resonance measured in a separate tube containing a solution of 0.5 g ¹⁵NH₄⁺¹⁵NO₃ in 3 ml D₂O/H₂O (Varian ¹⁵N standard 920 350-57). Each spectrum represents 120,000 pulse repetitions.

total yield of cells was a tightly packed pellet of about 10 ml. This material was divided into several batches, and both the cell pellets obtained by centrifugation and the growth medium were investigated using NMR.

In a first experiment about 2.8 ml of the packed cell slurry were deposited in a 12-mm NMR tube immediately after collection. A proton noise-decoupled ^{15}N -NMR spectrum of such a sample shows a group of four distinct resonances at -249.8 p.p.m., -253.8 p.p.m., -256.2 p.p.m. and -261.9 p.p.m., the amplitude of the signal at -253.8 p.p.m. being about ten times larger than that of the other resonances (Fig. 1b). No other resonances were observed in the spectral region from 30 to -470 p.p.m. To check for the possibility that some or all of the signals could arise from ^{15}N -labelled metabolites present in the growth medium external to the cell, a spectrum of the supernatant medium was recorded in the same conditions (Fig. 1a). A single signal was detected at -280.9 p.p.m., that is 19 p.p.m. more than the highest field signal arising from the cell pellet shown in Fig. 1b. This shows unequivocally that the signals in Fig. 1b arise from compounds within the cell.

In a second experiment, another batch of about 3 ml of the packed cell slurry was resuspended in the culture medium, and kept at 3°C for 9 d after collection. Thereafter the cells were separated by centrifugation, resuspended in distilled water, and left overnight at 10°C with continuous stirring. The cells were then centrifuged and transferred to an NMR tube as described above. The spectrum of such cold-aged, washed cells is shown in Fig. 1c. Only one signal was observed at -260.9 p.p.m., showing that at least three components present in the freshly collected cells (spectrum b) diffused out or were metabolised during the ageing period. Direct evidence for a metabolic inter-conversion comes from the observation that the strong line in spectrum c is in a spectral range which contained only a weak line in the freshly collected cells. Within the accuracy of the chemical shift determination it may well be that the signal in spectrum c and the line at -261.9 p.p.m. in spectrum b correspond to the same compound. Observation of resonances at 251.9 p.p.m., in the range of 145 – 155 p.p.m., and at 51.6 p.p.m. in the aqueous extract showed, on the other hand, that ^{15}N -metabolites leaked out during the wash.

At this stage the question arises as to the origin of the observed resonances. Overall, the frequency region in which the resonances are observed is typical for peptidyl amides. For example, the glycylglycine amide resonance is at -258.5 p.p.m. in the range $4 < \text{pH} < 10$, (ref. 10) and for a variety of other dipeptides and polypeptides the ^{15}N chemical shift typically varies from -270 to -230 p.p.m.¹¹. On the other hand, from chemical shift considerations the following representative compounds may be excluded: free amino acids (-290 to -350 p.p.m., excluding the ring nitrogens of histidine and tryptophan)¹¹, NH_4^+ (-354 p.p.m.)¹², $(\text{H}_2\text{N})_2\text{C}=\text{O}$ (~ -300 p.p.m.)¹³. The *Ustilago* ^{15}N signals are narrower and better defined than the resonances observed by Lapidot *et al.*¹⁰ in ^{15}N -labelled haemoglobin, which seems to be the only protein whose ^{15}N spectrum has been reported. Low molecular weight peptides exhibit relatively narrow ^{15}N resonances as exemplified by the natural abundance ^{15}N -NMR spectra of Viomycin and gramicidin S, a cyclopenta- and a cyclodecapeptide, respectively¹¹. The resonances shown in spectra b and c may thus quite likely arise from the low molecular weight peptide pool of the cell, intermediate in protein and peptide synthesis. In the growth conditions used here, *U. sphaerogena* is an active producer of the ferrichrome cyclohexapeptides, which contain a triornithyl sequence¹⁴. This suggests that the cells possess an active nitrogen metabolism in the region of the urea cycle so that it is not surprising that small ^{15}N -labelled metabolites should occur in sizeable concentrations.

The present study definitely establishes the suitability of ^{15}N -NMR as a non destructive tool for studying aspects of the cellular nitrogen metabolism, especially so since isotopic effects of ^{15}N , *vis-à-vis* the most abundant ^{14}N isotope, ought to be negligible. As with ^{13}C -NMR, improved instrumentation

and increased availability of labelled compounds should further enhance the appeal of ^{15}N -NMR for biochemical studies.

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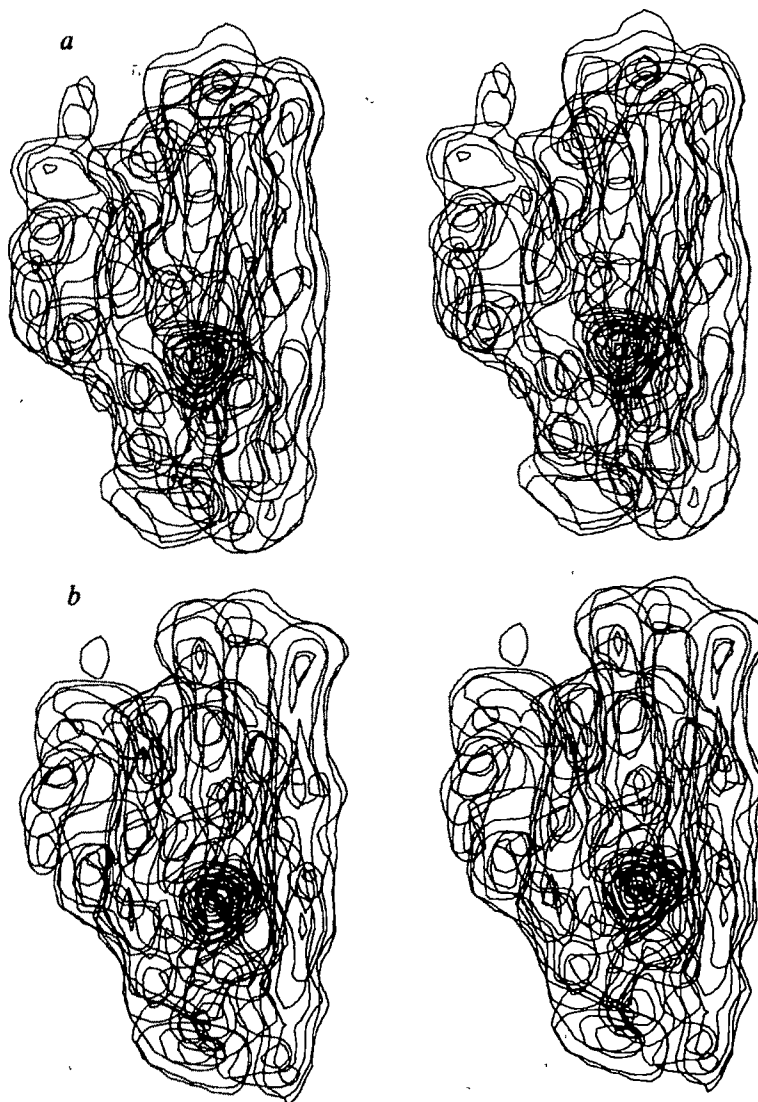
Quaternary and tertiary structure of haemerythrin

THE oligomeric protein haemerythrin is an oxygen-transport pigment found in erythrocytes of the coelomic fluid of certain invertebrates. It usually occurs as an octamer of molecular weight 108,000, in which each subunit contains two Fe atoms and reversibly binds one O_2 molecule¹. There is convincing evidence that myohaemerythrin, a monomeric protein found in the retractor muscles of the sipunculan worm *Themiste pyroides*, and the protomers of haemerythrin have quite similar tertiary structures^{2,3}. Consequently, the low resolution structure obtained for myohaemerythrin⁴ has been used to solve the structure of octameric haemerythrin by molecular search techniques.

Haemerythrin from the sipunculan *Phascolopsis* (syn. *Goldfingia*) *gouldii*⁵ is a hybrid molecule comprising five molecular variants, four of type A and one of type B, which differ only slightly in primary structure. Metazide-haemerythrin was dissociated and haemerythrin B monomers were isolated as described previously⁶. After purification by rechromatography, the monomers were reacted with excess cysteine ethyl ester to reconstitute octameric haemerythrin B (ref. 7). The protein was purified on Sephadex G-75 and concentrated by pressure ultrafiltration to give a 30 mg ml^{-1} solution. Tetragonal crystals were grown in sealed drops containing $5\text{ }\mu\text{l}$ of this solution and $2\text{ }\mu\text{l}$ of 2-methyl-2,4-pentandiol. The diffraction pattern from these crystals has Laue symmetry $4/\text{mmm}$ with reflections $00l$ extinct for odd l . The lattice constants measure $a=b=104.82\text{ }\text{\AA}$ and $c=54.08\text{ }\text{\AA}$. A more detailed description of the preparation of reconstituted octameric haemerythrin and of crystals grown from this material will be presented elsewhere.

The unit cell volume relative to the molecular volume of haemerythrin indicated that there must be two molecules

Fig. 1 Stereoplots of isolated electron density distributions (a) for a myohaemerythrin molecule and (b) for a haemerythrin protomer. The orientation for each corresponds to that of subunit 1 of Fig. 2. The myohaemerythrin map is a rotated version of one presented earlier⁴. The haemerythrin map is based on phases derived initially from the myohaemerythrin model and improved by a process involving the averaging of electron density related by non-crystallographic symmetry and the constraining of density in solvent regions to a constant level. The first contour level is at $0.53 \text{ e } \text{\AA}^{-3}$ for myohaemerythrin and at $0.51 \text{ e } \text{\AA}^{-3}$ for haemerythrin. Higher contours are drawn at intervals of $0.20 \text{ e } \text{\AA}^{-3}$. Density values are on a quasi-absolute scale and include the F_{000} contribution. Although these density distributions for myohaemerythrin and haemerythrin are similar, significant differences are evident. The most pronounced structural difference appears at the CD corner and must reflect the five residue deletion in this region of haemerythrin compared with myohaemerythrin. The increased density near the beginning of helix C in haemerythrin may represent the bulkier side chains of residues (Glu, Phe, Tyr, Asp) which replace myohaemerythrin residues (Ser, Glu, Val, Val) at positions 68–71 (G. L. K., J. L., Cote, and S. E. Ludlam, unpublished). On the other hand, apparent differences at the start of the A helix may only be due to excess density from a close lattice contact in the myohaemerythrin crystal. It is interesting that the density appended at the first bend in the N-terminal arm, which decreased during the course of myohaemerythrin refinement, is much reduced in haemerythrin. These and other seemingly significant differences in the two maps indicate that the bias towards myohaemerythrin in the initial phase information for haemerythrin has been largely overcome.



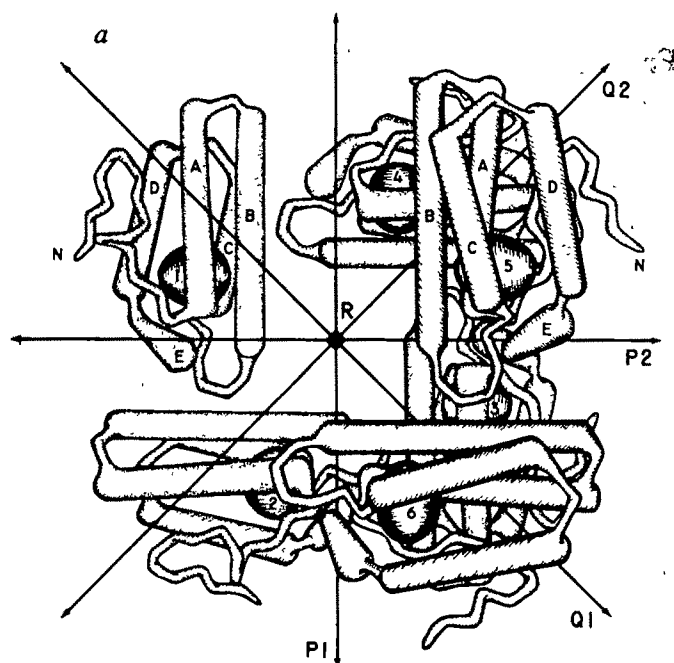
in the unit cell to give a solvent content in the range typical of protein crystals⁸. Placements of two molecules in space group $P4_222$ are incompatible with the molecular symmetry and dimensions suggested by previous studies of haemerythrin crystals^{9,10}. Instead, the data are readily explained by two octamers centred about the special positions $(0, 0, 0)$ and $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$ in space group $P422$. Since the cell is not centred, the two molecules must differ in orientation by a 45° rotation about the c direction. Pseudo eightfold symmetry observed in $hk0$ precession photographs is consistent with this view. Exact 422 (D_4) symmetry of haemerythrin B is therefore revealed by this elegant arrangement which, in addition, uniquely defines the relative disposition of the two protomers in the asymmetric unit.

X-ray diffraction data from $\text{CuK}\alpha$ radiation were collected using a diffractometer to 5.5 \AA resolution and corrected for absorption and the L_p factor. Initial phases for the observed structure amplitudes were calculated by Fourier inversion of a model structure composed from the electron density of isolated myohaemerythrin molecules appropriately oriented and positioned. An approximate orientation was determined by using the fast rotation function of Crowther¹¹ to correlate the observed intensity data with the squared transform of the electron density distribution in an isolated myohaemerythrin molecule. Attempts to ascertain the position using a translation function¹² were unsuccessful. Fortunately, a packing analysis limited the possible translational positions to three unique regions in the cell. Structure factors calculated from reoriented

myohaemerythrin molecules positioned at one of these possibilities satisfactorily matched the observed 20-\AA resolution data. This model was then improved by trial and error adjustment of the rotation and translation parameters in order to reduce the conventional R factor for data to 8-\AA resolution. Finally, the parameters were refined by a succession of fittings of the myohaemerythrin density to 5.5-\AA resolution Fourier maps of haemerythrin B based on phases from the current model. Details of the structure analysis will be presented elsewhere.

Several factors affirm the correctness of this structure solution. The final model structure gives an R value of 0.43 for all $1,141$ data. Packing contacts between subunits in this structure are stereochemically reasonable (Table 1) and consistent with biochemical evidence on possible subunit interfaces. Further credence is lent to the proposed structure by the strong density returned at expected iron sites in a Fourier synthesis of haemerythrin based on phases calculated from a myohaemerythrin model from which density for the dimeric iron centre had been removed. Also, the iron atoms of myohaemerythrin transform to positions close to multiples of $c/16$ which explains the great intensity of reflection $(0, 0, 16)$. Finally, Fig. 1 illustrates that the major differences in tertiary structures seen between these images of haemerythrin and myohaemerythrin correlate with the known differences in primary structure⁴.

The eight subunits of *P. gouldii* haemerythrin B are associated in 422 symmetry to form a molecule having approximate external dimensions of $75 \times 75 \times 50 \text{ \AA}$. The



quaternary structure is illustrated in Fig. 2. Each subunit is oriented with its long (pseudo-diad) axis roughly perpendicular to the fourfold rotation axis (R). The assembly consists of two layers, each a square comprising four protomers related by R in an end-side arrangement. Layers are related to one another by the four twofold axes ($P1$, $Q1$, $P2$, $Q2$) which lie normal to R in a plane between layers. This formation produces a channel along R which swells to a chamber in the centre of the molecule. The chamber is 30 Å in diameter and 15 Å high and is bounded

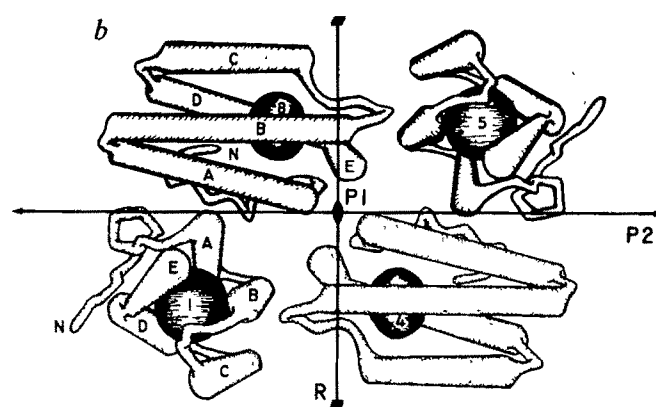


Fig. 2 Quaternary structure of haemerythrin B. Axes in a molecular coordinate frame are designated by $P1$, $Q1$, $Q2$ and R . For the molecule shown, diads $P1$ and $P2$ and the tetrad R are parallel to x , y and z , respectively, and intersect at $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$ of the crystallographic system. Subunits are drawn as schematic representations of the polypeptide chain. The amino-terminal arm is designated by N and helical segments are labelled $A-E$ in accordance with the notation for myohaemerythrin⁴. The dimeric iron centres are represented by the oblate spheroids on which subunit numbers appear. Subunits 1-4 are numbered anticlockwise about R starting from protomer 1 in the third quadrant of the lower layer. Subunits 5-8 are related respectively to 1-4 by diad $P1$. Subunit 1 as viewed in a corresponds to the electron density distribution of the haemerythrin protomer shown in Fig. 1. View a is along R and shows the four protomers (stippled) in the lower layer and two of the four upper layer protomers (hatched) related by diads P and Q to the former. View b shows four subunits, two from each layer, as seen from within the octamer along the diad $P1$. Figures drawn from computer-generated schematics.

Table 1 Subunit interfaces within and between octamers

Subunit pair and symmetry	Structural features with potential for contact	C_{α} pairs within 10 Å
R (1 \rightarrow 2)	BC (63-66) \rightarrow B (45-46, 48-50, 52-53, 56-57) BC (64-65) \rightarrow C (77, 81) E (107-108, 111-113) \rightarrow B (42, 45-46, 49-50, 53)	42
R (4 \leftarrow 1)	Reciprocal to R (1 \rightarrow 2)	42
$P2$ (1 \rightleftharpoons 7)	N, A (17-19) \rightleftharpoons N, A (17-19) N, A (17-20) \rightleftharpoons E (111-113)	22
$Q1$ (1 \rightleftharpoons 8)	N (8-12) \rightleftharpoons A (27, 30-31, 33-34, 38) N (11-12) \rightleftharpoons B (43, 46) A (19-20, 23) \rightleftharpoons B (46, 50) A (22-24, 26-27, 30-31) \rightleftharpoons A (22-24, 26-27, 30-31)	71
1 \rightleftharpoons 8' ($\frac{1}{2}, \frac{1}{2}, \frac{1}{2}$)	C (75, 78) \rightleftharpoons C (75, 78)	3
1 \rightarrow 6'' (0, 0, 0)	A, AB, B (36-41) \rightarrow N (1-3) CD (87-88) \rightarrow N (1-2) CD (87-88) \rightarrow D, E (103, 106)	15
1 \rightarrow 7'' (0, 0, 0)	N (1-3, 5) \rightarrow A, AB, B (38-41)	7

All of the subunit interactions encountered by one protomer from the octamer centred at $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$ are tabulated here. These include four intramolecular contacts due to symmetry operations of the diads, P and Q , and the fourfold axis, R , and three intermolecular lattice contacts with subunits of the other independent octamer. The specific subunits and symmetry operators designated in this table refer to those identified in Fig. 2. Subunit 8' is related to 8 by a unit translation in z and subunits 6'' and 7'' are related to 6 and 7 by a 45° anticlockwise rotation about $(\frac{1}{2}, \frac{1}{2}, z)$ and translation by $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$. The contacts made by a protomer from an octamer at (0, 0, 0) are identical to those of one at $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$ except for lattice contacts reciprocal to those cited as 1 \rightarrow 6'' and 1 \rightarrow 7'' above. Structural features are designated by helix and corner letters⁴ in the notation of Fig. 2 with sequence positions⁶ of specific residues in parentheses. The identification of residues with potential for intersubunit contact was made by noting pairs of C_{α} atoms from different subunits which lie within 10 Å of each other. The C_{α} positions were derived from a tentative model¹⁴ for the polypeptide backbone of this protein. Those C_{α} atoms (25, 28, 29, 35, 43, 47, 51, 54, 55, and 62) which met the 10 Å criterion for potential contact but which were shielded by more superficial residues were excluded in this tabulation. The resulting number of non-shielded intersubunit C_{α} pairs within 10 Å of one another is given in the third column for each interface. The closest approach of an intersubunit C_{α} pair is 4.1 Å within a molecule and also 4.1 Å between molecules. The closest approaches between C_{α} atoms of protomer 1 and those of 3, 5, and 6 are 18.4, 26.4 and 25.1 Å, respectively.

by helices A and B and the E helical stub. The channel is lined by B and C helices and has a limiting aperture 20 Å square. Other access to the central chamber may be possible through eight small openings alongside the *P* axes (Fig. 2b).

Of the six possible types of subunit interactions in an oligomer with 422 symmetry¹³, three occur in haemerythrin. One type is the heterologous association between adjacent protomers related by *R* in a layer. Each subunit participates in two such interactions. The major isologous interaction is that between neighbouring protomers related by *Q* axes. Less extensive interaction exists between adjacent protomers related by *P* axes. The subunit interfaces in these associations are characterised in Table 1 by reference to a tentative model¹⁴ for the polypeptide backbone of haemerythrin. Several studies have implicated the involvement of certain residues at subunit interfaces^{7,15-19}. In agreement, this model has Cys-50 located near both the *Q* and *R* interfaces, and has Tyr-18, His-34, four lysines, and several carboxylates also placed at contact surfaces. In contrast, Tyr-70 and the variable residues^{6,20}, with the exception of Glu-63 and Val-9, do not occur near subunit contacts. The relative importance of the *P*, *Q* and *R* interfaces in stabilising the octamer is suggested by the contact ratio 22:71:84. Moreover, since the ratio of intramolecular contacts to lattice contacts is 177:25, the molecular structure is probably little affected by lattice interactions; thus averaging of crystallographically-independent subunits is justifiable at this stage of refinement. A more detailed description of the structure will be presented elsewhere.

Before completion of this investigation, we were informed that the structure of *Themiste dyscritum* haemerythrin had been solved by a single isomorphous replacement. While this manuscript was in preparation, we received a preprint²¹ describing those results. The two independent analyses have revealed essentially identical structures.

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Functional equivalence of the two iron-binding sites of human transferrin

FLETCHER and Huehns^{1,2} presented evidence that the two iron atoms bound to the serum iron-transport protein, transferrin are not equally available for the synthesis of haemoglobin by immature red blood cells (reticulocytes). They hypothesised that functional differences between the binding sites may serve to regulate the distribution of iron within the body³. Various studies (cited in ref. 4) have been published since, some of which support and some of which contradict this hypothesis. We devised procedures⁴ to compare the availability of iron at each site of transferrin for reticulocytes *in vitro*, as well as to compare the availability of iron in diferric and monoferric transferrin. In the homologous system of rabbit reticulocytes and rabbit transferrin, there was no functional difference between the two sites of diferric transferrin or between diferric and monoferric protein. In the heterologous system of rabbit reticulocytes and human transferrin, however, a difference between the sites of diferric transferrin and between diferric

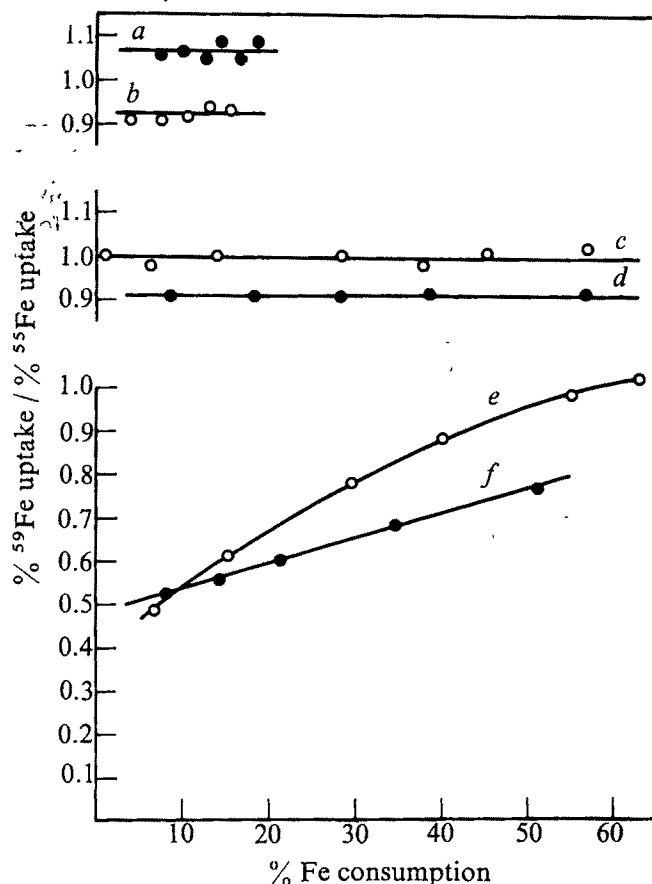
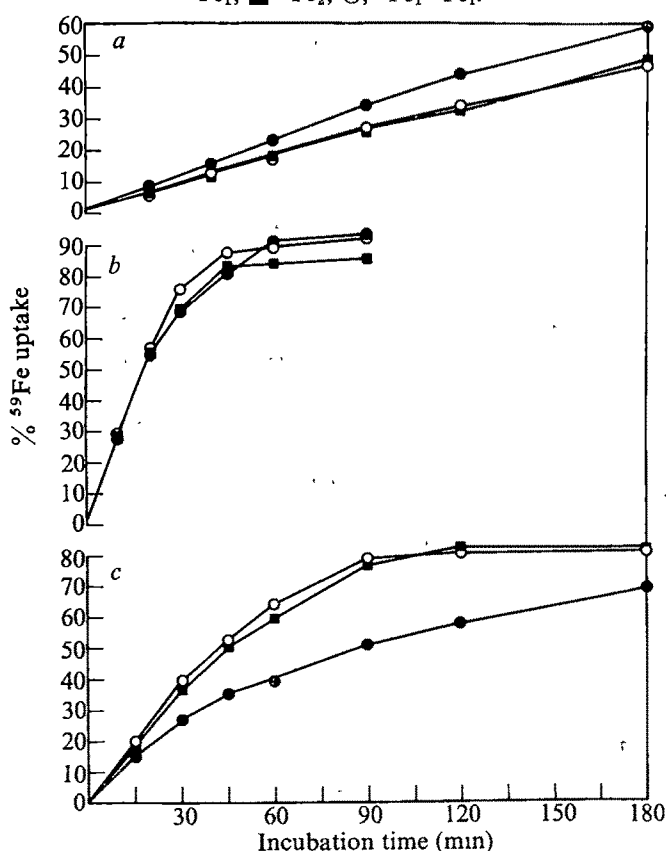


Fig. 1 Ratio of the uptake of ⁵⁹Fe and ⁵⁵Fe in double incubation experiments. *a* and *b*, Human cells plus human protein; *c* and *d*, rabbit cells plus human protein. To increase the fraction of iron consumed, the concentration of transferrin in experiments with human reticulocytes was 1-3 that used previously in experiments with rabbit reticulocytes⁴. Haematocrits in incubation mixtures ranged from 25 to 50%. In the first incubation the ⁵⁹Fe consumed was: *a*, 20%; *b*, 59%; *c*, 22%; *d*, 39%; *e*, 25%; *f*, 46%. In experiment *a* blood from a patient with thalassaemia intermedia was used in the first incubation, and from a patient with haemoglobin C disease in the second. In experiment *b* each incubation made use of blood from different patients with sickle cell anaemia. The reticulocyte counts in the human blood ranged from 9 to 18%. Total incubation times in the second experiment were *a*, 120; *b*, 150; *c*, 35; *d*, 22; *e*, 60; and *f*, 32 min. In experiments *e-f* the incubation medium contained bovine serum albumin at a concentration of 2 mg ml⁻¹ before addition of cells. In experiments *a* and *b* albumin was omitted. Experiments *c* and *e* come from ref. 4.

and monoferric transferrin was observed. Those studies have now been extended to the homologous system of human reticulocytes and human transferrin. We found no difference in availability of iron at the two sites of human transferrin and only a slight difference in the availability of iron in monoferric and diferric protein.

To assess the relative availability of iron at the two binding sites, the following procedure was used⁴. Transferrin was treated with a quantity of ^{59}Fe -nitrilotriacetate sufficient to saturate 115% of the specific binding sites, and the excess iron was removed by gel filtration through Sephadex G-25 equilibrated with the saline incubation medium. After adding glucose to a concentration of 1 mg ml^{-1} , the ^{59}Fe -saturated protein solution was incubated with reticulocytes until 20–59% of the iron had been consumed by the cells. A quantity of ^{59}Fe -nitrilotriacetate calculated to be 10% in excess of that needed to replace consumed ^{59}Fe was then added to the supernatant incubation solution. After standing for 30 min at 20°C and 30 min and 37°C , the solution was passed through a column of Sephadex G-25 to separate unbound ^{59}Fe . The eluate was concentrated by ultrafiltration to approximately its original volume and glucose was added to a concentration of 1 mg ml^{-1} . This doubly labelled protein solution was then incubated with fresh cells and the ^{59}Fe and ^{55}Fe uptakes were measured. If site A were a better iron donor than site B, ^{59}Fe would be removed preferentially from site A in the first incubation and ^{55}Fe would preferentially occupy site A in the doubly labelled material. Therefore, a greater fraction of ^{59}Fe than of ^{55}Fe should be consumed in the second incubation. Alterna-

Fig. 2 Comparison of iron-donating abilities of monoferric and diferric transferrin. Blood from a patient with sickle cell anaemia (11% reticulocytes) was used for the upper experiment, in which the incubation mixture contained 0.13 mg transferrin per ml and had a haematocrit of 38%. Qualitatively similar results were obtained with blood from patients with iron deficiency anaemia (9% reticulocytes) and pernicious anaemia (12% reticulocytes). All incubation solutions contained bovine serum albumin (2 mg ml^{-1}) before adding cells. The experiments with rabbit cells have been described previously⁴, and are shown here for comparison, *a*, Human cells plus human protein; *b*, rabbit cells plus rabbit protein; *c*, rabbit cells plus human protein. Transferrin: ●, $^{59}\text{Fe}_1$; ■, $^{59}\text{Fe}_2$; ○, $^{59}\text{Fe}_1$ $^{55}\text{Fe}_1$.



tively, if the sites function equivalently, the same fraction of each isotope would be consumed in the second incubation. To compare the availability of iron in monoferric and diferric transferrin, monoferric ^{59}Fe -transferrin obtained by isoelectric focusing was brought to saturation with either ^{59}Fe or ^{55}Fe and uptake of ^{59}Fe from the monoferric and both diferric species was compared.

Results of the double incubation experiments are shown in Fig. 1. In both homologous systems (*a-d*) the ratio of the fractions of each isotope consumed is within experimental error of unity throughout each experiment, clearly indicating that one site of transferrin is not a better donor than the other. With rabbit reticulocytes and human transferrin (*e* and *f*) there is a preferential uptake of ^{59}Fe in the second incubation, as expected if one site is a better donor. Furthermore, the ratio of uptakes approaches unity with increasing total iron consumption since all of each isotope is potentially consumable. (Curves E and F are not expected to be identical because the fraction of iron consumed in the first incubation was not the same in each case.)

In Fig. 2 the availabilities of iron from monoferric and diferric transferrin are compared. Iron from monoferric human transferrin was slightly but consistently more available to human reticulocytes than iron from diferric transferrin in three different experiments, only one of which is shown. It should be noted, however, that since diferric transferrin carries twice as much iron as monoferric transferrin, it provides more total iron per unit time than monoferric transferrin. In the case of rabbit cells and rabbit protein, each iron atom of the monoferric and diferric species seemed to be equally available to reticulocytes. In the heterologous system of human protein and rabbit cells, monoferric transferrin was a somewhat poorer source of iron than diferric transferrin.

During our experiments we abandoned the use of albumin in the incubation medium when we became aware of its iron content. When analysed by atomic absorption spectroscopy, a solution of crystalline bovine serum albumin (10 mg ml^{-1} , Schwarz-Mann) contained $0.2\text{--}0.35 \mu\text{g Fe per ml}$. This would have been enough to affect the results of experiments comparing monoferric and diferric transferrin, were the iron available to the transferrin. Therefore, we studied the kinetics of iron uptake from monoferric human transferrin by rabbit reticulocytes in the presence or absence of albumin, or in the presence of albumin from which more than 90% of the iron had been removed by passage through a column of Chelex 100 (BioRad) equilibrated with 0.1 M sodium acetate, $\text{pH } 5.5$. No consistent effect of albumin on the rate of iron uptake was observed, so we cannot rule out the possibility that iron may have been transferred from albumin to transferrin in our conditions. Nevertheless, we think this unlikely in the absence of an iron-complexing agent to mediate transfer⁵.

Our results in Fig. 1 are inconsistent with the hypothesis of Fletcher and Huehns³. Experiments in support of this hypothesis are those of Awai *et al.*^{6,7} who studied iron uptake by rat tissue *in vivo*. The discrepancy between these results and ours may simply reflect the difference in species. We cannot however, account for the results of Awai *et al.* by a simple mathematical model. For example, consider the following experiment of theirs. Blood rich in reticulocytes was incubated with serum, in which the transferrin had been 90% saturated with ^{59}Fe , until only 35% of the iron remained. Assuming the greatest possible site preference, the remaining transferrin contained no ^{59}Fe at site A while site B still bound 70% of the ^{59}Fe originally present there. This was mixed with serum brought to 35% saturation with ^{55}Fe , so that sites A and B should each be 35% occupied by ^{55}Fe . In the resulting preparation, only ^{59}Fe should be present at site A, while a 2:1 ratio of ^{59}Fe to ^{55}Fe should be at site B. Even if a tissue has an absolute preference for site B, therefore, it should only consume a 2:1 ratio of ^{59}Fe : ^{55}Fe . Yet it was reported that this preparation delivered a 50:1 ratio of ^{59}Fe : ^{55}Fe to rat liver parenchymal cells (Table 1, in ref. 6).

It may be significant that in most ferrokinetic studies in man, 95–98% of the plasma radioiron clearance can be accounted for by a single rate constant^{8,9}. If the tracer is bound randomly to the sites of transferrin, this would indicate that the sites are at least kinetically, and very likely functionally, equivalent in their iron-donating properties.

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Wavelength dependence of the bandwidths of visual pigment spectra

DARTNALL¹ proposed that the normalised absorption spectrum of any vitamin A₁-based visual pigment, when plotted on a frequency scale, could be fit to a standard shape, independent of its spectral absorbance maximum (λ_m). The existence of a standard shape implies that all pigments have the same band width. Dartnall devised an extremely useful nomogram so that given the λ_m of a pigment one could determine the entire shape of its main absorption band. A similar, slightly broader nomogram has been found for vitamin A₂-based pigments^{2,3}.

The validity of the idea that there are standard, λ_m -independent band shapes for all visual pigments, however, has been questioned^{4,5}. A clear trend has developed: spectra of blue-absorbing pigments tend to be broader than the nomogram, whereas red-absorbing pigments display spectra narrower than the appropriate nomograms. For example, the spectrum of the 432-nm frog cone pigment seems to be broader than the A₁ nomogram⁶. The action spectra of the 350-nm ultraviolet pigment and the 440-nm violet pigment in the moth *Deilephila elpenor* are clearly wider than the nomogram⁷; that of the 350-nm pigment is in turn considerably broader than that of the 440-nm pigment. Among the narrow, long wavelength spectra are those of the 620-nm tadpole pigment⁸, the 560-nm chicken cone pigment⁹, and the 625-nm goldfish cone pigment⁹. Smith and Pokorny⁵ have found that the nomogram does not describe the spectra of the long wavelength human cone pigments. In view of these findings, we investigated the theoretical guidelines governing visual pigment band shapes.

Several studies^{10,11} have described the spectral properties of the visual pigments in terms of those of two main classes of polyene molecules, cyanines and carotenoids. Cyanines, whose

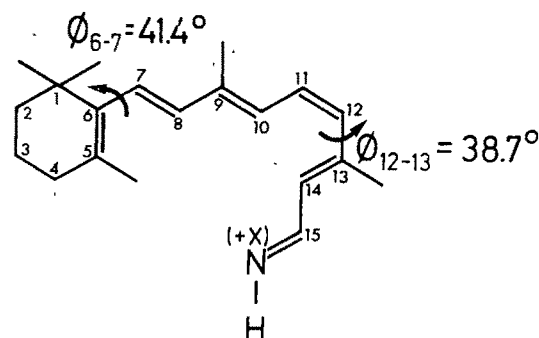


Fig. 1 11-*cis* retinal (protonated) Schiff base. X, Fraction of protonation $0 \leq X \leq 1$, as explained in the text. Values of the dihedral angles ϕ are from Gilardi *et al.*²³

π electrons are essentially completely delocalised, have approximately equal bond lengths and narrow bandwidths (about $1,000 \text{ cm}^{-1}$). In carotenoids, the π electrons are not completely delocalised; there is considerable single bond–double bond alternation and bandwidths are broad (about $6,000 \text{ cm}^{-1}$)¹². As a result of their bond alternation, carotenoids absorb at considerably shorter wavelengths than cyanines of comparable length. The amount of bond alternation also affects the width of the absorption bands¹³. If a given electronic transition causes a large displacement of the equilibrium nuclear configuration along a particular normal coordinate, a vibrational progression due to that normal mode will appear in the spectrum: the larger the displacement, the greater the extent of vibrational broadening. In polyenes, the first excited state is one of little bond alternation¹². Thus excitation of carotenoids involves a large displacement of the equilibrium nuclear configuration and a correspondingly broadened absorption band. In the cyanines, bond alternation is already indistinct in the ground state and so excitation results in only small displacements in the equilibrium nuclear configuration and little vibrational broadening.

In visual pigments, retinal is bound by way of a Schiff base linkage to the protein. The Schiff base, at least in vertebrates, seems to be protonated¹⁴ (for a review of the evidence, see ref. 4). There is a large red shift from the spectrum of free retinal to that of rhodopsin and other visual pigments. Calculations¹⁵ using the Pariser–Parr–Pople¹⁶ (PPP) technique show that this spectral shift can be associated with the formation of the protonated $\text{C}=\text{N}^+$ bond. The resulting chromophore has an increased degree of π electron delocalisation and a corresponding decrease in bond alternation. The process involves, in effect, conversion of the chromophore from a carotenoid (high degree of bond alternation, short λ_m) to a cyanine-like (little bond alternation, long λ_m) molecule. Differences in absorption maxima between pigments are attributed to secondary interactions with the protein^{15,17}. The expectation, based on this analogy with polyenes, is that a decrease in bandwidth should accompany any increase in λ_m (ref. 4). It would be of interest to determine whether these qualitative considerations are borne out by detailed theoretical calculations.

Table 1 Calculated and experimental wavelengths, calculated bandwidth parameters, and experimental bandwidths for several model compounds

Molecule	$(\text{CH}_3)_2\text{N}-(\text{CH}=\text{CH})_4-\text{CH}=\text{N}^+(\text{CH}_3)_2$	$\text{O}^--(\text{CH}=\text{CH})_4-\text{CH}=\text{O}$	11- <i>cis</i> retinal (at the crystal conformation of Fig. 1)	11- <i>cis</i> retinal Schiff base (at the crystal conformation of Fig. 1)
Reference	20	20	21	22
$\lambda_{\text{calc}}(\lambda_{\text{exp}})(\text{nm})$	623 (625)	540 (548)	339 (365)	347 (350)
$R(\text{\AA})$	0.0213	0.0358	0.0980	0.0957
$\Delta\nu_{\text{exp}}(\text{cm}^{-1})$	700	1,100	7,200	6,200

Calculations carried out in the standard PPP–MO technique¹⁶ in which carbon–carbon and carbon–protonated nitrogen overlap parameters were fit to match the spectra of the cyanine series $\text{N}^+=\text{C}_{2n+1}\text{N}$ (ref. 20). The carbon–unprotonated nitrogen overlap integral was chosen to reproduce the experimental spectrum of an 11-*trans* retinal Schiff base²². The carbon–oxygen overlap integral was chosen to reproduce the $\text{O}=\text{C}_{2n+1}\text{O}^-$ spectra²⁰. Details elsewhere (A. D. G., B. H., and T. G. E., unpublished).

Table 2 Positions and bandwidth parameters for the λ_m in the protonated Schiff base, calculated at the crystal conformation of 11-*cis* retinal (Fig. 1)

Fraction protonation	λ_m (nm)	$R(\lambda_m)(\text{\AA})$
0.0	347	0.0957
0.2	367	0.0901
0.4	416	0.0833
0.6	498	0.0746
0.8	600	0.0631
1.0	670	0.0485

McCoy and Ross¹⁸ have found a direct correlation between a theoretically-derived parameter R and the shapes of electronic bands in aromatic systems. The parameter R relates the displacement of the equilibrium value of the bond lengths to the intensity distribution within the vibrational envelope of a transition. With Δr_j equal to the excitational change in the j th bond length, they consider the quantity

$$R = \left(\sum \Delta r_j^2 \right)^{1/2} \quad (1)$$

Here Δr_j can be calculated from the ground and excited state bond orders using the standard¹⁹ relationship between bond length r_j and bond order P_j ,

$$r_j = r_j^0 - \xi_j P_j \quad (2)$$

where r_j^0 is the ideal single bond length and ξ_j is the empirically determined slope.

Parameters for the calculations carried out here were obtained by fitting theoretical transition energies to experimental absorption maxima for a number of polyenes related to the visual chromophore. Table 1 compares calculated and experimental wavelengths for some of these model molecules. The fit of the absorption maxima is quite good. Moreover, we note that the trend in the calculated values of R parallels the experimental trend in bandwidths; R is large for carotenoids and small for cyanines.

Calculations involving the visual pigment chromophore were carried out on the π electron system consisting of atoms 5–16 in Fig. 1. To investigate the bandwidth parameter over a large range in λ_m , we varied the fraction of protonation on the nitrogen, x , from zero to one (Fig. 1). Suzuki *et al.*¹⁵ have shown that the amount of charge on the terminal nitrogen can control π -electron density in the bonds and thus the amount of bond alternation. Varying the amount of protonation mimics the more complicated chromophore-protein interactions which cause the observed variations in λ_m . Results for the protonated Schiff base (Table 2) show that the experimentally observed trends are reproduced by our calculations: R decreases as λ_m increases. Moreover, the numerical values of R in the range of visual pigment absorption maxima are intermediate between those of cyanines and carotenoids but closer to the latter. This is consistent with the observation that the width of the nomogram is 4,300 cm^{-1} .

In conclusion, we have shown that the (protonated) Schiff base model of visual pigments predicts bandwidths which are wavelength dependent. Moreover, the observed trend in pigment bandwidths can be understood in terms of the qualitative considerations presented here. Thus, it is not surprising that it is impossible to fit the spectra of all pigments to a standard band shape. Rather, any nomogram should be applicable only to a limited range of absorption maxima; in the case of the Dartnall nomogram, this range is from about 470–530 nm.

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X-ray diffraction study of molecular structure and conformation of mezerein

THE crystal structure of one of the toxic components of the plant *Daphne mezereum*, mezerein, has been solved, unequivocally showing the molecular conformation in the solid state. Extracts from this plant have long been known to contain curative components, and have been used extensively in folk medicine in several countries¹. Mezerein has attracted attention for its anti-leukaemic activity against P-388 and P-1210 leukaemias in mice².

Many esters of phorbol (Fig. 1), which is closely related chemically to mezerein (Fig. 2), have also shown strong biological effects. Although these esters are reported to be extremely carcinogenic³, 12-hydroxy-daphnetoxin (Fig. 2) has no such activity. Furthermore, a range of esters of 12-hydro-daphnetoxin has significant antitumour activity⁴.

Because mezerein is the 5-phenyl-2,4-pentadionate ester of daphnetoxin, it would be interesting to know whether the attachment of this side chain changes the conformation of daphnetoxin or whether the biological activity is due solely to the added side chain. Also a knowledge of the architecture of this and related compounds will facilitate a systematic search for the biological systems affected by and the mode of action of chemical induction and inhibition of cancer.

Using a crystal of mezerein three-dimensional X-ray diffraction data were collected and reduced using conventional methods. The crystal belonged to the orthorhombic space group $P2_12_12_1$ with unit cell dimensions $a=13.45 \text{ \AA}$,

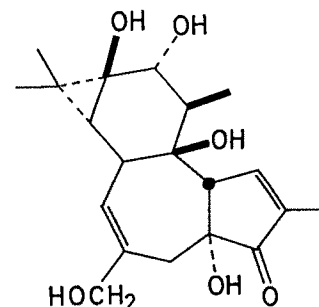


Fig. 1 Phorbol (the crystal structure of which has been solved by Brandl *et al.*⁷).

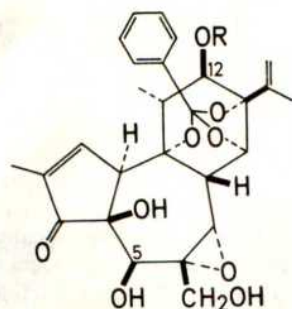


Fig. 2 12-Hydroxy-daphnetoxin (R = H), or mezerein (R = COCH-CHCH-CHC₆H₅).

$b=21.60 \text{ \AA}$ and $c=11.56 \text{ \AA}$, and contained one molecule per asymmetric unit. The structure was solved using direct methods and is at present refined to an R value of 0.131, using a full matrix least squares technique and isotropic temperature factors (details will be given elsewhere).

The molecular structure of the compound looks extremely compact, except for the 5-phenyl-2,4-pentadionate side chain. The fused-ring systems consisting of three, five, six and seven-membered rings form a cluster of roughly spherical shape (Fig. 3). The conformation of the central part of the molecule compares with that of 12-hydroxy-daphnetoxin^{5,6}. The cyclohexane ring is in a slightly twisted boat conformation. The structure of the cycloheptane ring can be regarded as that of a basal plane consisting of five carbon atoms of which the carbon-carbon bond involved in the epoxide ring is a member. At an angle of 110° to this plane the plane defined by the two bonds shared with the five-membered ring and the six-membered ring is attached.

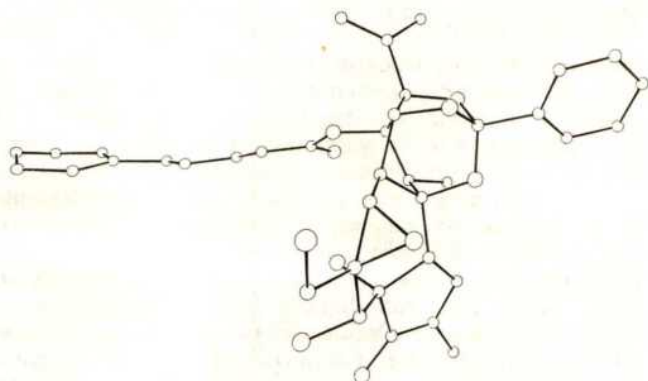


Fig. 3 A computer-generated drawing of the molecular structure of mezerein based on crystallographic data.

There seems to be conformational identity between the molecular structures of mezerein and that of 12-hydroxy-daphnetoxin tribromoacetate in the crystalline state⁶. In addition, due to the rigidity of the molecular framework caused by the attachment of various ring systems to the cycloheptane ring, it is most unlikely that the solution conformations are significantly changed.

We can thus conclude that the observed antitumour activity is somehow connected to the added ester at position 12. As Kupchan *et al.* pointed out⁴, the affixed ester may be involved in processes associated with cell wall penetration. In this connection it might be pointed out that the diterpene is in its fully extended form. An important question remains: at which step in the cellular metabolism is the toxic effect of mezerein and its analogue exerted, and which of the functional groups are active?

The crystal of mezerein used in this study was supplied by A. Groth and R. Söderquist, FOA, Stockholm, Sweden.

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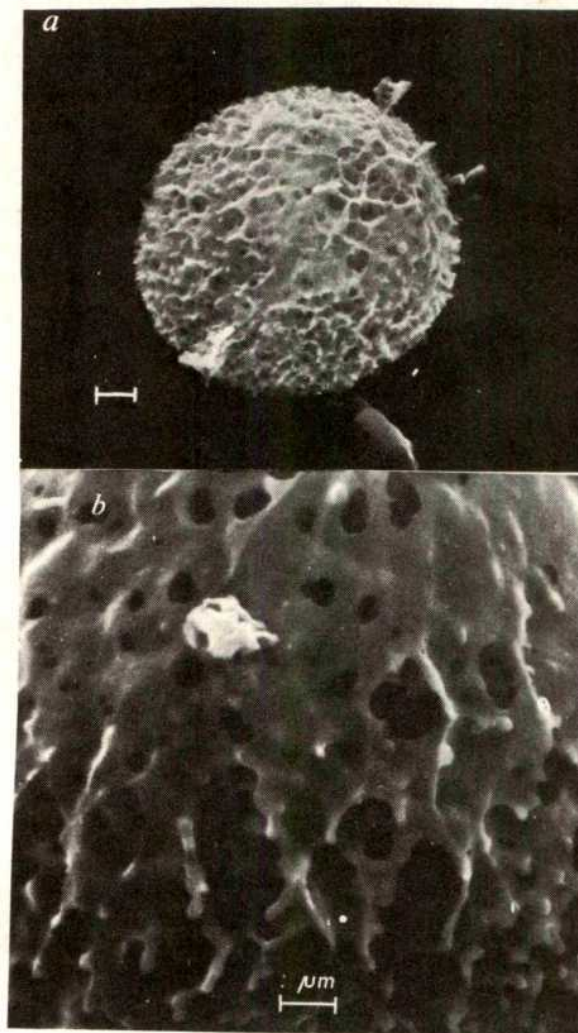
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Use of macromolecules in microparticles

POLYMERS of acrylamide, dextran, Agarose, and their derivatives have been used widely as matrices for covalent immobilisation of enzymes, for example, for biospecific affinity chromatography¹. Macromolecules can also be trapped in acrylic polymers, when added to the monomer solution before polymerisation²⁻⁴. Spherical polymeric particles are produced by homogenising the monomer solution with a suitable detergent in an organic solvent or mixture, in such a way as to produce a water-oil emulsion before polymerisation. The size of the polymeric particles will be directly proportional to the size of the droplets of the monomeric solution in the organic phase, giving a narrow size-distribution of the particles. The mean diameter can be varied greatly by changing the homogenising conditions. Hitherto, the aim has been to prepare particles with a mean diameter of 100-250 μm , to ensure a high flow rate when they are packed in columns. We have now found, however, that macromolecules trapped in the particles, retain their biological properties, and that therefore, microparticles

Fig. 1 Scanning electron micrographs. *a*, Polyacrylamide particle obtained by bead polymerisation; *b*, detail of microparticle. Bars, 1 μm .

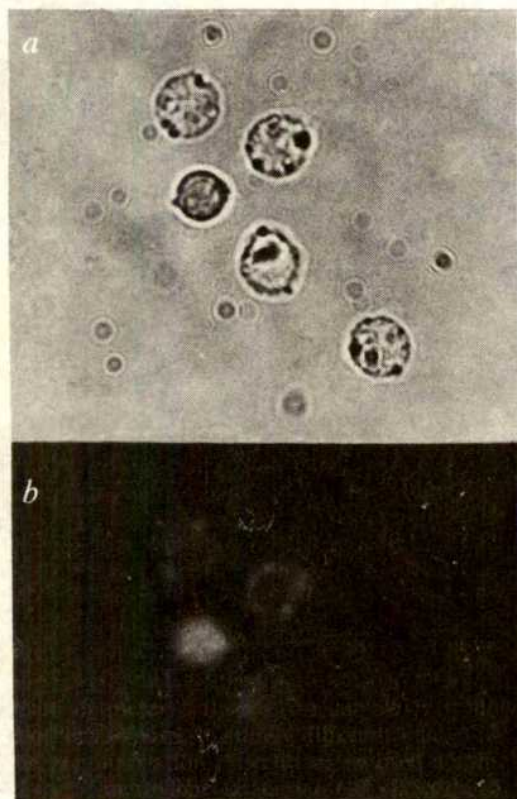


with mean diameter $<10\ \mu\text{m}$ can conveniently be used for several other purposes in widely different fields.

In the polymerisation procedure, we used acrylamide (1.5 g) and N,N' -methylene-bisacrylamide (0.5 g) in 25 ml of phosphate buffer, 0.005 M, pH 7.5, in which the protein (10–100 mg ml^{-1}) was dissolved. The catalyst system, consisting of ammonium peroxodisulphate (10 μg in 50 μl) and N,N,N',N' -tetramethyl-ethylenediamine (50 μl), was added to the monomer solution immediately before the homogenisation in toluene-chloroform (4 : 1, 300 ml). The detergent (Pluronic F68, Wyandotte Chemical Corp., Wyandotte, Michigan, 0.25 g) was dissolved in the organic phase. After the polymerisation, which was completed in about 10 min, the particles obtained were thoroughly washed with water and centrifuged. A more detailed description of the preparation procedure and the characteristics of the microparticles will be given elsewhere.

The amount of protein trapped, determined after acid hydrolysis, is directly proportional to its concentration in the monomer solution. Protein not trapped in the particles is found both in the water phase and the first washings. The polymeric network trapping the proteins is very porous, facilitating good penetration of the particles by reasonably large molecules, as is evident from Fig. 1. The equilibrium with the entrapped macromolecules is, therefore, reached very rapidly. Different macromolecule–ligand interactions, for example drug–protein or protein–protein interactions, can thus be easily studied using the microparticles. The material to be studied is suspended in an appropriate buffer, in which the particles with entrapped macromolecules will form a separate phase which can be isolated by centrifugation. During prolonged incubation in the buffer only insignificant numbers of macromolecules leak out of the particles. The particles can also serve as a matrix for different macromolecular reagents, for example in radioimmunoassays.

Fig. 2 Photomicrographs of fluorescein-labelled microparticles, containing concanavalin A, interacting with human erythrocytes, at pH 7.4 in 0.9% NaCl with 0.001 M CaCl_2 , 0.001 M MnCl_2 , 0.001 M MgCl_2 and 0.02% merthiolate. The photographs show the same cells in *a*, visible light; and *b*, blue light (about 490 nm).



The binding properties of the macromolecules trapped in acrylic polymers are unchanged. Thus, for instance, the binding of salicylic acid and warfarin to albumin is the same when immobilised in macroparticles as in equilibrium dialysis, with albumin in free form in the buffer. This enables binding studies to be performed in a suspension of particles, which can be isolated by centrifugation after brief incubation, obviating time consuming and laborious methods, such as equilibrium dialysis with semipermeable membranes. The use of microparticles containing albumin should greatly simplify the analysis of the drug-binding capacity of individual patient sera, enabling an individualised dosage regimen, using potent protein-bound drugs, to be achieved. Methods are being developed for this purpose, based on the competitive protein-binding principle.

A remarkable feature of the entrapped macromolecules is their availability on the surface of the microparticles, enabling them to interact with biological cells. Figure 2 shows how microparticles containing 2% concanavalin A (con A) and labelled with fluorescein isothiocyanate, have formed complexes with human erythrocytes. Particles not containing any con A will not show any significant affinity for the erythrocytes. In preliminary experiments it has also been shown that microparticles containing protein A from *Staphylococcus aureus*, known to react with the Fc part of IgG molecules⁵, are completely retained (at pH 7.4, in 0.1 M NaCl) on a Sepharose column, to which normal human immunoglobulin is covalently bound. Apparently, the polyacrylamide network allows sections of the protein to stick out of the microparticles, and to react with cells or Sepharose particles, but is compact enough to keep other sections of the protein molecules fixed in the particles.

The discovery that the macromolecules are accessible to specific membrane structures on the cell surface, opens up wide perspectives for the separation and characterisation of cells with specific surface structures. The separation of cells has hitherto been based mainly on centrifugation in density gradients, and results in bands of cells of the same density.

In a cell population of the same density, however, different cells can have different surface properties. Advantage has been taken of this to separate cells on, amongst others, nylon nets, to which biospecific molecules are bound⁶, or on biospecific Sepharose columns⁷. The microparticles, in which specific macromolecules are trapped, can be used for the same purposes. After a group of cells has been separated in a density gradient, the microparticles can be used to change the density of cells bearing the receptor for which the macromolecules have affinity. Repeated gradient centrifugation will then separate these cells. This general principle can be used in several different applications.

The microparticles can easily be labelled, with different fluorescent reagents, for example, large amounts of which can be bound to inactive macromolecules trapped in the particles. Combined with specific cell-markers (antibodies, agglutinins), such microparticles can be used to detect specific cell membrane structures using fluorescence microscopy. Such an application can be used in different cell studies, as well as for diagnostic purposes, the importance of which cannot yet be foreseen.

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matters arising

GFA protein in C-6 glioma *in vitro*

BISSELL *et al.* reported a 40-fold increase of glial fibrillary acidic (GFA) protein by radioimmunoassay in buffer extracts of C-6 glioma cells maintained in organ culture, compared with the same cells in monolayer and suspension culture¹. Quantitative data using buffer extracts should be interpreted with caution, since they may indicate differences in solubility rather than in total amounts. GFA protein is only partially soluble in water^{2,3}, and the factors regulating the ratio between water-soluble and insoluble fractions are still poorly understood. Solubility in buffer is greatly increased by *in situ* proteolysis⁴, and may be seen to vary considerably in different regions of the brain, even when a relatively insensitive assay such as double immunodiffusion is used.

- ¹ Bissell, M. G., Eng, L. F., Herman, M. M., Bensch, K. G., and Miles, L. E. M., *Nature*, 255, 633-634 (1975).
- ² Eng, L. F., Vanderhaeghen, J. J., Bignami, A., and Gerstl, B., *Brain Res.*, 28, 351-354 (1971).
- ³ Dahl, D., and Bignami, A., *Brain Res.*, 57, 343-360 (1973).
- ⁴ Dahl, D., and Bignami, A., *Biochim. biophys. Acta*, 386, 41-51 (1975).
- ⁵ Miles, L. E. M., Bieber, C. P., Eng, L. F., and Lipschutz, D. A., *Radio-immunoassay and Related Procedures in Medicine*, 1, 149-164 (International Atomic Energy Agency, Vienna, 1974).

BISSELL *ET AL.* REPLY—Bignami¹ raises the question whether our reported data on the quantitative increase of GFA protein in C-6 glioma cells *in vitro*² might not indicate differences in solubility rather than in total amounts. We welcome this opportunity to stress that the GFA protein estimations in our study were determined only on the phosphate buffer-soluble fraction. We have of course long been aware³ of the existence of water-soluble and insoluble GFA protein fractions, and we agree that the chemical relationship between the two and the

protein and of the stability and immunological identity of the purified and impure antigen, followed by a discussion on the soluble compared with insoluble GFA protein fractions, is to appear soon⁵. Contrary to Bignami's experience, our purified GFA protein standard (prepared from frozen human multiple sclerosis plaques) did not change in two-site IRMA immunoactivity for at least six months. In our report all samples were measured using the same standard.

Stanford University School of Medicine

- ¹ Bignami, A., *Nature*, 257, 827 (1975).
- ² Bissell, M. G., Eng, L. F., Herman, M. M., Bensch, K. G., and Miles, L. E. M., *Nature*, 255, 633-634 (1975).
- ³ Eng, L. F., Vanderhaeghen, J. J., Bignami, A., and Gerstl, B., *Brain Res.*, 28, 351-354 (1971).
- ⁴ Bissell, M. G., Rubinstein, L. J., Bignami, A., and Herman, M. M., *Brain Res.*, 82, 77-89 (1974).
- ⁵ Eng, L. S., Lee, Y.-L., and Miles, L. E. M., *Anal. Biochem.* (in the press).

Table 1 Solubility of GFA protein in different regions of the brain indicated by the immunodiffusion titre with GFA protein antiserum

	Water-soluble fraction	Urea-soluble fraction (first extract)
Cerebral cortex	1/4	1/4
Cerebral white matter	< 1*	1/32
Brain stem	1/8	1/16

Bovine brains were frozen at the slaughterhouse and GFA protein was extracted in 0.05 M sodium phosphate buffer, pH 8.0, with and without 6 M urea (w/v, 1:4).

* Precipitin lines were not observed against undiluted GFA protein antiserum unless the well was filled 2-3 times with the white matter extract.

As Table 1 shows, the white matter contains larger amounts than the cerebral cortex, which agrees with its relatively larger content of astroglia. However, the reverse is true for the water-soluble fraction, that is, only small amounts are extractable in buffer from the white matter compared with the cortex.

An additional point is that the radioimmunoassay for GFA protein⁵ is difficult to reproduce, since no information is given on the preparation of the standard. Our experience with micro-complement fixation indicates that GFA protein not only precipitates from the standard solution kept at 4 °C or frozen, but also that in these conditions the immunological activity per unit protein steadily decreases in the high-speed supernatant (100,000g for 1 h).

A. BIGNAMI

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factors influencing their relative concentrations are still poorly understood. To assign, therefore, any special significance to either is premature. On the other hand, while we cannot exclude the hypothesis that variations in solubility could have played some role in determining minor deviations in the quantitative data obtained in our study, the overall reproducibility of the assays and the fact that they so unequivocally confirm previous observations on the same system using an immunofluorescence technique⁴ make it unlikely that solubility differences would account in any significant measure for the striking increases observed. The relevance of Bignami's table in relation to our report is questionable because the heterogeneity of astroglial cell populations that prevail in the different regions of the brain does not apply to the homogeneous cloned cell system used in our study.

A description of the properties of the two-site IRMA assay for the GFA

Tropomyosin and actin

BECAUSE of changes introduced before publication, Parry's letter¹ was not quite that to which my reply² was addressed. I wish to point out that his suggestion that tropomyosin would present pseudo-equivalent aspects to all seven actins is simply a restatement of my original hypothesis³. This hypothesis is strengthened by the observation of several charged groups in the sequence⁴ repeating at exactly 77 residues. The computed coordinates of these show that some could lie at the correct positions to interact with one strand of actin.

W. LONGLEY

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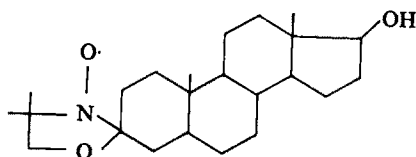
- ¹ Parry, D. A. D., *Nature*, 256, 346 (1975).
- ² Longley, W., *Nature*, 256, 347 (1975).
- ³ Longley, W., *Nature*, 253, 126-127 (1975).
- ⁴ Stone, D., Sodek, J., Johnson, P., and Smillie, L. B., *Proc. 9th FEBS Meet., Budapest* (in the press).

PHA and lymphocyte membrane fluidity

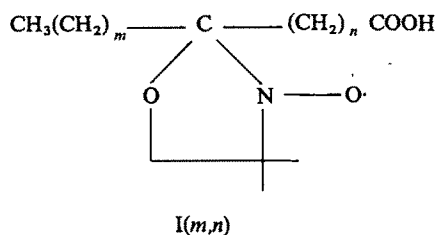
BARNETT *et al.*¹ reported that mitogenic lectins induce changes in the fluidity of lymphocyte membranes, which can be detected by means of the spin label electron spin resonance (ESR) technique. This finding, if substantiated, would be

of great importance in understanding the processes leading to mitosis. Considerable doubt has, however, been cast on the interpretation of Barnett's results, by the fact that the spin label used has since turned out to be a mixture of three compounds². Consequently, an independent study has been undertaken to examine the effects of the mitogen phytohaemagglutinin (PHA), using several different spin labels that probe different regions of the membrane.

The cells examined were human tonsil lymphocytes, separated (>90% purity) by teasing through a mesh and suspended in Hank's medium. ESR measurements were made using a Varian E-9, X-band spectrometer, equipped with a variable temperature accessory and a dual cavity operating in the H_{014} mode. Quantitation of the spectra was aided by the use of a Nicolet 1020A signal averager. Spin labels were purchased from the Syva Corporation, Palo Alto, California. These were the androstane label



and the stearic acid labels



where $m = 1$, $n = 14$; $m = 5$, $n = 10$ and $m = 12$, $n = 3$. All these labels are known to incorporate into the lipid regions of membranes. Two different methods were used to label the cells, namely deposition of the label on to the walls of a flask by evaporation of an alcoholic solution, and addition of $1 \mu\text{l}$ of a 10^{-1} M solution of label in ethanol to 1 ml of cell suspension. In both cases, cells were incubated with the label for 15 min at 37°C . They were then washed three times with medium, to remove free label and resuspended. A third method was also tried for label I ($m = 12$; $n = 3$), this being dissolved in an aqueous solution of sodium carbonate before addition to the cell suspension; however the method of introduction of label seemed to have no influence on the results obtained. Introduction of a spin label into the cells also had no detectable effect on their integrity. The PHA used

was Wellcome purified reagent. The optimum mitogenic dose for this material was found to be $1 \mu\text{g ml}^{-1}$ and this is the concentration used, except in one experiment where a higher dose ($10 \mu\text{g ml}^{-1}$) was used.

The androstane label and the stearic acid label I ($m=1$; $n=14$) both gave weakly immobilised, three-line spectra when added to the tonsil lymphocytes. The ratio of the heights of the three lines, which gives a measure of the mobility of the label³, was found to be unaffected by incubation with PHA for times up to 90 min. The label I ($m=5$; $n=10$) gave a complex spectrum showing both weakly and strongly immobilised components, possibly a result of some localisation of the polar nitroxide group in a hydrophilic region. Although difficult to analyse, this showed no detectable change with PHA. The label I ($m=12$; $n=3$) gave a strongly immobilised 'powder' spectrum when incorporated in the cells. From the separation of the extrema, an order parameter, S , was calculated⁴. This was determined with an accuracy of $\pm 1\%$ by means of the Nicolet 1020A. No change in the value of S could be detected, up to 90 min after the addition of PHA at 37°C . Use of a higher concentration of PHA also showed no effect on the value of S . When spectra were recorded at $21 \pm 1^\circ\text{C}$, the values of S were 0.678 ± 0.004 in the absence of PHA and 0.674 ± 0.007 in the presence of PHA. When measurements were made at 35°C , the fluidity of the membrane was increased and the value of S correspondingly reduced to 0.57 ± 0.01 .

In one experiment, cells were suspended in medium containing serum, in order to promote growth. As previously reported⁵, however, it was found that after introduction of spin label the observed spectrum was due primarily to label complexed with serum albumin.

Investigations into the fluidity of erythrocyte membranes in myotonic muscular dystrophy⁶ suggest that the spectral amplitude ratio A/B is more sensitive to change than the order parameter, S .

Consequently the present spectra were also analysed in terms of this parameter, but in no case could any change as a result of PHA be detected.

The present results indicate that PHA has no demonstrable effect on the fluidity of human tonsil lymphocyte membranes and it is unlikely that other human lymphocytes will behave differently. An alternative interpretation of the findings of Barnett *et al.*¹ must therefore be found. Since individual labels, in the mixture they used, would give differing values for the order parameter, the changes following mitogenic stimulation are consistent with a change in the relative proportions of the three labels present in the membranes. The cause of such a change could be as important as the apparent change in fluidity to which it was ascribed and this is now being investigated.

I thank Drs M. Moore and M. R. Potter of the Immunology Section for help and advice. The work was supported by the MRC.

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¹ Barnett, R. E., Scott, R. E., Furcht, L. T., and Kersey, J. H., *Nature*, **249**, 465-466 (1974)

² Barnett, R. E., Scott, R. E., Furcht, L. T., and Kersey, J. H., *Nature*, **255**, 262 (1975)

³ Smith, I. C. P., in *Biological Applications of ESR*, (edit. by Swartz, H. M., Bolton, J. R., and Borg, D. C.), 483-539 (Wiley-Interscience, New York, 1972).

⁴ Seelig, J., *J. Am. chem. Soc.*, **92**, 3881-3887 (1970)

⁵ Landsberger, F. R., Paxton, J., and Lenard, J., *Biochim. biophys. Acta*, **266**, 1-6 (1971).

⁶ Butterfield, D. A., Chesnut, D. B., Roses, A. D., and Appel, S. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 909-913 (1974)

Chandler Wobble and viscosity in the Earth's core

AN addition is necessary to the references given by Dr Rochester¹. The (l_1, m_1) he is using are those of the reference² given as (1950a) on p.257 of *The Earth*, fourth edition.

BERTHA JEFFREYS

160, Huntingdon Road,
Cambridge CB3 0LB, UK

¹ Rochester, M. G., *Nature*, **255**, 655 (1975)

² Jeffreys, H., *Mon. Not. R. astr. Soc.*, **110**, 460-466 (1950); *Collected Papers*, **3**, 547-553 (Gordon and Breach, London, 1974).

Erratum

In the original contribution by Rochester¹ the following text corrections should be made: para 1, line 12, $(\dot{l}_1^2 + \dot{m}_1^2)/\omega^2$. and para 2, line 17, $\gamma = (\dot{l}_1^2 + \dot{m}_1^2)^{1/2}/\omega$.

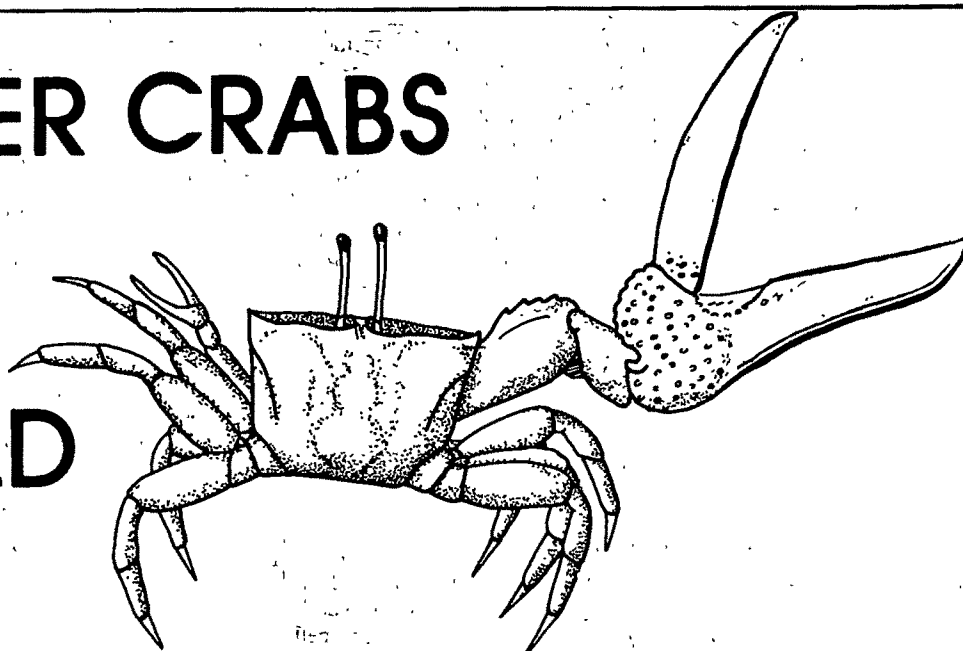
¹ Rochester, M. G., *Nature*, **255**, 655 (1975)

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reviews

WAVES of various kinds are present in the atmosphere and the study of their generation, propagation and interaction is of great importance to meteorologists and aeronomers. Compressibility and buoyancy effects due to the action of gravity on vertical density gradients provide the main restoring forces for waves of comparatively short wavelength, with Coriolis effects increasing in importance with horizontal wavelength and becoming dominant for planetary-scale waves. Hydromagnetic effects due to the presence of the Earth's magnetic field complicate wave propagation in the electrically-conducting ionosphere.

Numerous observations of atmospheric waves are now available, thanks to the introduction of a variety of new techniques, including radar and acoustic echo sounders. The first of the three books listed above is an excellent systematic account of meso-scale and small-scale waves dominated by compressibility and buoyancy. It starts with an historical survey and particularly lucid discussions of the basic equations of fluid dynamics and of various theoretical concepts required in the study of wave propagation. Some boundary value problems arising in the analysis of wave-propagation in non-uniform media are then considered. Chapter 6 is largely concerned with various stability problems and the theory of the generation of atmospheric gravity waves, and chapter 7 with the theory of dissipative effects. Observations of gravity waves and infra-sound and

Air waves

R. Hide

Waves in the Atmosphere: Atmospheric Intra-Sound and Gravity Waves—Their Generation and Propagation.

By Earl E. Gossard and William H. Hooke. Pp. xv+456. (Developments in Atmospheric Science, volume 2.) (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) \$64.95; Dfl 156.00. *Atmospheric Waves.* By Tom Beer. Pp. xvi+300. (Adam Hilger: London, October, 1974.) £16.00. *The Upper Atmosphere in Motion: A Selection of Papers, with Annotation.* (Geophysical Monograph No. 18.) By C. O. Hines and Colleagues. Pp. xii+1,072. (American Geophysical Union: Washington, DC, 1974.) n.p.

considerations of energy sources are presented in chapters 8–10, which include some particularly valuable case studies. Waves in the upper atmosphere are the subject of the eleventh and final chapter. This carefully prepared and well illustrated book can be highly recommended to graduate students and research workers in atmospheric physics.

The book written by Beer cannot, unfortunately, be highly recommended, owing to its many errors and muddled presentation of key ideas and results. This is a great pity because the publishers have gone to a great deal of trouble to produce

a handsome and well-illustrated volume, and there is certainly a need for a good treatise on the material covered, which includes treatments of waves on all scales.

Hines' book is altogether different from the other two, and it is by no means clear for whom the book is intended, apart from those specialists who may wish to possess in one volume a collection of excellent papers by one of the pioneers of research on waves in the upper atmosphere and his collaborators, together with a very personal and somewhat idiosyncratic commentary on each paper by the author. In the introduction to the book the author states that "... although I have by no means ignored my fellow professionals in making my commentary ... my thoughts were primarily with the student reader and my design was to provide him or her with a full view of the sort of workings that go on behind the development of a field. Scientific convention normally bars such exposure from orderly textbooks, but this unconventional format provided me with an unusual opportunity—one that normally arises in direct contact with students—and I have not hesitated to exploit it, hopefully for the benefit of students elsewhere". Some future historian of aeronomy will doubtless acknowledge the enterprise of the American Geophysical Union in making this material available, but few science students will have the time to pursue these fascinating byways. □

THERE are already available several comprehensive accounts of nuclear structure, but this volume is assured of a worthy place by reason of its clarity of exposition, attention to detail and coverage of the most recent advances.

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topics of the semi-empirical mass formula and infinite nuclear matter. There is a detailed account of the

Nuclear structure

P. E. Hodgson

Nuclear Structure. By William F. Hornyak. Pp. xii+605. (Academic: New York and London, July 1975.) \$49.50; £23.75.

single-particle model, showing how a simple one-body potential is able to account for so many features.

Substantial chapters follow on the individual particle or shell model and on the collective models. That on the shell model contains an

account of the coupling schemes and the general symmetry classifications, together with intermediate coupling and the cluster model. The account of collective models covers the classical liquid-drop model, the vibrational states of spherical nuclei and the collective states of deformed nuclei. Two concluding chapters are devoted to the electromagnetic interactions of nuclei and β decay, and there are appendices on angular momentum coupling and on the Wigner–Eckart theorem.

The level of presentation is intended to be that appropriate to a second-year graduate student, but the book will also be found valuable by senior undergraduates and established physicists. □

Proton-Transfer Reactions

Edited by E. F. CALDIN and V. GOLD, F.R.S.
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Many of the fundamental concepts of chemistry – from the tunnel effect in chemical kinetics to catalysis by enzymes – owe their recognition or formulation to the study of proton-transfer reactions. The subject has been developed in extensive researches by many workers, and most notably by Professor R. P. Bell, whose book *The Proton in Chemistry* has become a modern classic of science. The present volume, dedicated to Professor Bell, aims to complement his book by providing a coordinated set of studies in depth on some of the central problems and growing-points of proton-transfer reactions, written by a group of leading workers in the field

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Dynamic molecules

Molecular Reaction Dynamics. By R. D. Levine and R. B. Bernstein. Pp. vi+150. (Clarendon: Oxford; Oxford University Press: New York, November 1975.) £5.00.

THE last fifteen years have seen rapid progress in one of the most fundamental areas of chemical research—the awakening of our understanding of how reactions occur at the microscopic level. Indeed much of this work is so recent that an adequate grasp of the field can only be acquired after extensive perusal of the original literature and the relatively limited number of research reviews. It is for this reason that the appearance of Levine and Bernstein's *Molecular Reaction Dynamics* is particularly welcome at this time.

The book is directed at 'newcomers' to the field and contains material which has been presented orally to advanced undergraduates and, presumably, graduate students. The contents are organised into six chapters which make up a total of some 30 sections, most of which are subdivided further. The avowed aim is to provide a general feeling for the whole subject area rather than supply extensive detailed knowledge, and as such the volume is, as it sets out to be, much more of a 'course book' than a reference work. Chapter 1 serves to whet the appetite for the study of molecular dynamics, after which the logic is to initiate a detailed consideration of molecular collisions (chapter 2) as a prelude to an account of how scattering phenomena can be related to collision dynamics (chapter 3). This leads to a consideration of the trajectories across potential energy surfaces, the theoretical calculation of reaction rates, the relationship between micro- and macroscopic observations, the partitioning between the different forms of energy in the products (chapter 4) and a survey of the different forms of molecular energy transfer (chapter 5). The climax of this development is then experienced in chapter 6 in which the previous extensive preparation is applied to the experimental study and discussion of a series of chemical systems exhibiting respectively different types of interaction between the reacting species. The various sections of the book are adequately cross-referenced forwards and backwards.

The method of presentation is such that maximum value can only be derived from the text by reading it cover-to-cover a number of times, and a newcomer would only acquire a real understanding of some of the

mathematical material after a prolonged study of the relevant published literature. An annoying and distracting feature, which seems to be characteristic generally of 'beam' publications, is the extensive use of footnotes, often containing asides or amplifications of the discussion, which could readily be either omitted or incorporated into the main text. Apart these objections, however, the book undoubtedly satisfies the need for a readable and comprehensive guide to this important area of study at an attractive price. J. F. J. Todd

Numbers

Transcendental Number Theory. By Alan Baker. Pp. x+147. (Cambridge University Press: London, April 1975.) £4.90; \$13.95.

A NUMBER is said to be algebraic if it is a root of a non-zero polynomial in one variable, the coefficients of which are integers, and to be transcendental otherwise. It is well-known that (in a measure theoretic sense) almost all numbers are transcendental, but to demonstrate that a specific number is transcendental is never trivial. The methods for solving this problem constitute transcendental number theory.

Professor Baker's tract is an account of the advances in this subject during the last decade. A short description of the origins of the theory is followed by an amalgam of the author's award-winning papers (Fields Medal) on linear forms in logarithms with some very interesting applications both in transcendence theory and in such other areas as Diophantine equations and Gauss's conjecture on discriminants of class number one. Further topics include Schmidt's theorem on simultaneous approximation of several algebraic numbers by rationals, Mahler's classification of transcendental numbers and Shidlovsky's theorem on algebraic independence.

The proofs of the major theorems are presented in a very economic style and can be fully understood only by students with a plentiful supply of pencil, paper and time. For such serious students the book will form an admirable introduction to this difficult field of research, being in addition a concise source book for the major additional developments and outstanding problems. Those wishing to obtain an appreciation of the scope of this field will also gain much from the more descriptive parts of the work. All readers of this timely and welcome tract will benefit from the appraisal of a pre-eminent contributor to the field. D. A. Burgess

Urban environmental management

Urban Environmental Management. By Brian J. L. Berry and Frank E. Horton. Pp. xv+425. (Prentice-Hall: Englewood Cliffs, 1974.) \$14.95.

BRIAN BERRY and Frank Horton have discovered a new way to produce large text books—the 'text with integrated readings'. They now follow their *Geographical Perspectives on Urban Systems* with a new text on urban environmental management. They produce a cross between a reader and a traditional textbook by using contributors' papers, as in the former, but integrating them with judicious introductory and linking texts of their own. This makes it possible to be the first in the field with a new book. But does it work?

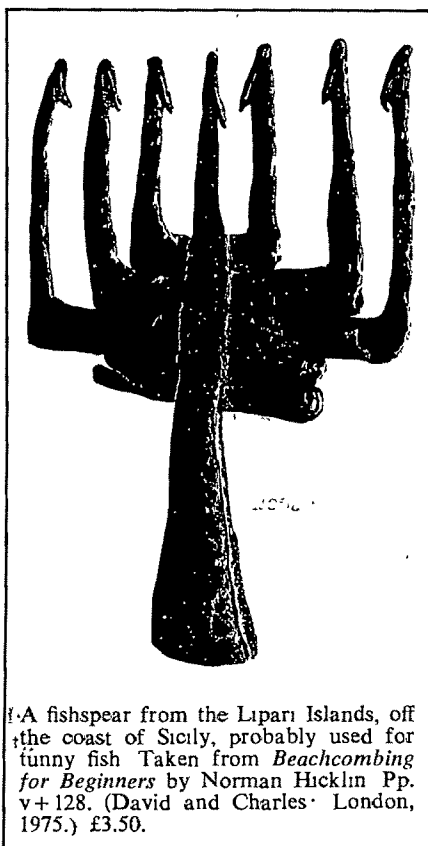
Although many of the contributing authors are first class, the present book is very uneven. Many of the articles used first appeared in that excellent journal *Science* and serve as thorough introductions. Most of the rest are either university discussion papers or government papers. These often contain masses of information, ranging in content, for example, from 'Generation of Solid Wastes from Five Major Sources in 1967' (covering the whole of the USA with no reference cited) to 'Licensed Swine Feeders in Northeastern Illinois' (reference given this time). Not surprisingly, it is very difficult for the reader to get all this into perspective. Absolute figures for 1967 do not say whether the problem is getting worse and if so at what rate.

Successive groups of chapter are concerned with environmental beliefs, environmental impacts of urbanisation, air, water, solid wastes, noise, the relationship between environmental pollution and health, and government programmes. Quite an agenda. The student is introduced to most of the basic elements of the various sectors, but he will sometimes, one suspects, have to be wary of the arguments put. For example, it is argued that particulate concentration increases with city size on the basis of a regression of the geometric mean of the pollutant against the log of the population, January temperature and the number of iron and steel production workers. Presumably, these were the best of the variables tried; the R^2 was 0.26. There are other examples of feeble analysis.

So the student gets his text a couple of years before he can have a 'proper' one, and lots of research workers who need a quick introduction to an important new field, can get it. But the price is fairly high. The quality of what is on offer varies tremendously, and the authors/editors have not always helped. They do indicate where a new reading starts, roughly, but not where it finishes, so it is not always

clear who is responsible for a piece of text—a bad principle to introduce. Many, if not most, of the entries in a long list of references are not actually cited in the text, and so the reader is not provided with an adequate guide to the literature. All in all, it may have been better for Berry and Horton to have been more patient before using their obvious skills in producing a conventional text.

A. G. Wilson



A fishspear from the Lipari Islands, off the coast of Sicily, probably used for funny fish. Taken from *Beachcombing for Beginners* by Norman Hicklin Pp. v+128. (David and Charles: London, 1975.) £3.50.

Insect muscle

Insect Muscle. Edited by P. N. R. Usherwood. Pp. xi+621. (Academic: London and New York, May 1975.) £14.50; \$38.25.

PHYSIOLOGISTS disagree as to whether diversity is a subject worthy of study in its own right but none would dissent from the view that a correct choice of experimental material is important for the investigation of basic biological phenomena. It has been amply demonstrated that insect muscle can provide many such opportunities. By contrast, it cannot be claimed that the study of insect muscles has yet made any important contributions to applied entomology. Workers who are interested in practical control measures can omit this book from their reading for the time being. It might become important introductory material for them in 10 years time when other aspects of insect physiology are better understood. Otherwise, however, this is a useful and timely book which should be read by all

general physiologists interested in the problem of muscular contractility.

Elder starts with a good review of all the significant, fine structural aspects of insects muscles. Finlayson deals with the difficult subject of development and degeneration, but in a book of this sort the articles should be more than a catalogue of original sources of information and should bring out points of significance. In fact, many of the articles meet this criterion.

On neuromuscular physiology, Osborne gives a good discussion of ultrastructural aspects and Usherwood and Cull-Candy provide a review of the difficult field of the identity of transmitters; their summary tables on the action of various chemical substances will be much used for reference. Piek, dealing with ionic and electrical properties, suggests that a highly active Na-K exchange pump operates in the extensive transverse tubular membrane, that this membrane is permeable to chloride and that this leads to a concentration of NaCl in the transverse tubular space which is high enough to produce a significant liquid junctional potential at its opening.

Aidley gives a short review of excitation-contraction coupling and mechanical properties, which is adequate in view of the fact that few significant differences have been discovered between insect and vertebrate muscles. Tregear presents a major review of the use made of a fibrillar muscle preparation for the elucidation of basic mechanisms of mechanochemical transduction; insect muscle had made important contributions to this general problem. Crabtree and Newsholme, on metabolism, return to an emphasis on diversity. Whereas the basic molecular mechanism for contraction seems to be the same for all muscles, differences in the type of fuel used for metabolic generation of ATP reflect the adaptation of the tissue to the needs of the whole animal, which are different for different insects. The article contains a lot of information and an interesting discussion of regulatory mechanisms and the nature and role of reversible mitochondrial shuttles. Hoyle, on neural control, is concerned with the programming of the output of motor neurones. Insect movement provides several well defined patterns of coordination, the analysis of which offers hope for a better understanding of the organisation of the central nervous system. This is an active and significant area of research. The last chapter, by Miller on visceral muscles, is a tasty *hors d'oeuvre* which, I hope, will stimulate the appetite of comparative physiologists for a field that cannot fail to integrate several aspects of insect biology.

The book is well produced and Professor Usherwood deserves our gratitude for undertaking the editorial tasks.

J. W. S. Pringle

Belts of ignorance

The Geology of Continental Margins. Edited by Creighton A. Burke, Charles L. Drake. Pp. xiii+1009. (Springer: Berlin and New York, 1974.) DM85.30; \$34.80.

FOR 600 million years the level of the North American continent as a block seems to have deviated by less than 60 m from the levels of the surrounding oceans. Yet during that time the Atlantic and Indian oceans came into being, the shape of the Pacific must have changed considerably, and the entire ocean floor has been renewed possibly twice or thrice. There must, therefore, be some mechanism which maintains a balance between the capacity of the ocean basins and the quantity of water available to fill them. It is very difficult at the moment to see what this mechanism may be. That is but one of the problems to which this book contributes.

The book provides a first rate summary of what is known about the continental margins—belts of ignorance as they have been termed—which extend sinuously across the Earth's surface for 350,000 km. These remarkable regions, which were necessarily ignored by the early landbound geologists and which have to some extent been left on one side by the recent rapid advances in our knowledge of the deep oceans, remain of critical importance.

It has long been known that sediments produced by the wearing away of continents contribute to the continental shelves, but what has become clear only in recent years is the effective way that such deposits are retained near the continents and the small extent to which they contribute to the sediments of the deep oceans. We now know of some of the ways in which sedimentary material is clawed back to form, once more, parts of a modified continental crust.

This book provides both a synoptic view of the margins of the present day continents and a conspectus of more general matters, such as bathymetry, the transition from continent to ocean, recent sedimentation, and the deformation of continental margins, all of which are treated in a score of contributions. The latter parts of this 1,000-page book deal with problems of the small ocean basins, such as the Black Sea or the Mediterranean, and with questions concerning ancient continental margins, the subject of a dozen or so papers. There is no index, but an excellent concluding article by the editors puts matters in perspective in an effective fashion, and the lay-out, consisting as it does partly of articles grouped under general headings, and partly of articles dealing with geographical regions, makes reference not too difficult. Nonetheless, in my opinion an index would have been a most useful addition.

One of the main virtues of this book arises as a result of the factual information that it contains, arranged as it is in a readily accessible fashion. But perhaps of greater importance are the discussions of still wider problems, which come up time and again. Why are continental margins situated where they are? How is it that, despite the great changes in the shape and distribution of oceans, the water rarely overflows on to the continents by more than a few tens of metres? One gets a vivid impression of the way in which geophysical evidence as to the structures of recent continental margins, supplemented by drilling, is bringing our knowledge to a point from which direct comparisons can be made with the remnants of ancient continental margins whose structures have been gradually pieced together by two centuries of geological enquiry.

From an economic point of view one is left with an intriguing question: whether petroleum may be present within the continental rise, the deepest lying part of the continental margin. Existing technology could probably be developed to recover hydrocarbons, should they be present, from the sediments of the continental rise, though these are covered by as much as 2–3 km of water.

All in all this is a first class book which does justice to the immensity of its subject.

J. Sutton

Aggression

Determinants and Origins of Aggressive Behaviour. (New Babylon: Studies in the Social Sciences, vol. 22.) Edited by Jan de Wit and Willard W. Hartup. Pp. xiv+623. (Mouton: The Hague and Paris, 1974.) Dfl.84.

THE papers in this collection “were prepared for” or, alternatively, “grew out of” a NATO conference held in Monaco two years ago. The first and more modest estimate of their origins is that of the editors, whose thoughtful concluding paper testifies to the limits of growth that can be expected from such a conference. The 45 papers embrace a good range of current experimental approaches to the understanding of aggressive behaviour: the perspectives of ethology, physiological psychology, psychogenetics, social psychology, psychometry, pharmacology, psychopathology and anthropology are all represented to some degree.

In such a jamboree, coherence is almost inevitably missing. At least, the editors make little pretence of finding it: their own consideration of the effects of domestic punishment on children's aggression in other situations involves alarming discrepancy across just 11 pages; and at no point does their discussion owe anything to the one experimental report in the volume which explicitly concerns

Miller's displacement model of aggression. Perhaps out of deference to the many other assembled experts, few of the authors show much proselytising zeal for their own perspectives, and so the clarity born of dramatic confrontation is absent: even such giants as Berkowitz and Eibl-Eibesfeldt make few claims to each other's territory.

Nor are the editors helped by the largely unsurprising, if occasionally contradictory nature of the results which the papers report. Disagreements as to the effects of social isolation, of parental socialisation practices and of television violence rumble through these papers; but there are no dramatic breakthroughs. Perhaps the papers which best combine topicality and substance are those of Christiansen and Hutchings: by using both cohort twin-study and adoptive methodology the partial heritability of criminality is reasserted; and Christiansen's data show that offences against the person are no exception.

Looking to the future, the editors finally opt to stress the need for “field studies”. But it seems an open question whether the armchair would not initially be a better location than the field if the laboratory does have to be left unmanned for a time. Both Hinde and the editors provide cogent illustrations of the diversity of aggressive behaviour and of the problematic nature of the concept of ‘aggression’ itself. Such a position doubtless reflects current academic wisdom in this area. But other papers—especially that by Oweus—note the way in which conceptually distinguishable ‘types’ of aggressive behaviour tend to appear together: across individuals, it can often be said that some are more generally ‘aggressive’ than others.

Trait and factorial approaches to aggression—especially if informed by the experimental and psychogenetic input which they have lacked in the past—would certainly help to settle some of the current confusion as to how many types or dimensions of aggressive behaviour are worth recognising. It is idle to rehearse for long ‘the multifactorial nature of aggression’ without using all appropriate methods of asking what the factors are and what proportion of natural variance they could possibly explain.

The general dimension of ‘aggressive behaviour/disorder’ that seems so commonly in epidemiological work on individual differences remains largely unexplored in these papers; as do the processes of competition and conflict in human social hierarchies that might have had more relevance to NATO and more chance of incorporating the ethological notion of the normality of ‘aggression’. The volume is a competent and catholic sampling of the experimental studies of aggression conducted during the early 1970s; but it yields no very satisfying vision of the future.

C. R. Brand

Not quite the whole truth

Neutron Stars, Black Holes and Binary X-ray Sources. (Astrophysics and Space Science Library, Vol. 48.) Edited by Herbert Gursky and Remo Ruffini. Pp. xii+441. (Reidel: Dordrecht and Boston, Massachusetts, 1975.) Dfl. 135; \$54.00 cloth; Dfl. 95; \$38.00 paper.

THE study of neutron stars and black holes, believed to be associated with many of the binary X-ray systems now discovered in space, has certainly progressed to the point where there is a need for a book pulling together the current state of the art in both observation and theory, and this volume fills the role, on the whole, admirably. The introduction by the editors provides a historical overview that is both comprehensive and a pleasure to read, but then the sense of anticipation with which the reader begins on the book proper is somewhat dashed by the terrible standard of English which he encounters: "Supernovae are the explosion of a star. This awesome and exciting realisation came about at the turn of the century from the following sequence of observations", and so on. In any multi-author book (this one is based on a session of the annual meeting of the AAAS held in February 1974), active editing is necessary to achieve a uniformly clear level of communication, and it is quite possible to achieve this without losing the character of the remarks of individual contributors. Am I alone in pleading for the abandonment of the Royal "we" in single author papers and in objecting to such circumlocutions as while analysing the data on directions or that paper the author also came across information...? When he could say "I found"? Even one of the editors is guilty of jargon disease in an individual contribution, and as the price of books increases such irritations seem to become more noticeable and less excusable.

In terms of scientific content, however, this is a very good book. The emphasis on physical interpretation rather than on the erudite mathematics of general relativity is welcome, and although there is some rejection of ideas, especially from the different authors discussing aspects of the standard binary model, this helps in providing a better insight than the model was discussed on a 'one-and-or-all' basis in a chapter of its own. One of the most remarkable features of the family of binary models is the patience with which a few dedicated theorists have worked out details for different mass ratios and configurations, even in the days before observations of binary X-ray sources gave an

impetus to work of that nature.

The chapter on observational properties of pulsars features the most complete table of pulsar parameters I have ever seen, covering 105 of these objects, and provides an early attempt to use this wealth of data for statistical investigations of pulsar properties. While the book was in preparation the discovery of a pulsar in a binary system opened a new phase in pulsar studies, and although E. J. Groth, the author of this section, might not agree it is in some ways convenient that his review should thus neatly tie up the pulsar package in the light of discoveries made before that identification.



Scanning electron micrograph of head of nematode worm, *Enoplus* sp.. Taken from *The Biology of Free-living Nematodes* by Warwick L. Nicholas. Pp. viii+219. (Clarendon: Oxford; Oxford University Press: London, August 1975.) £8.25.

The binary pulsar discovery paper is reproduced in an appendix, together with other contemporary and historic papers, a convenience for those readers too lazy to leaf through bound copies of journals in libraries. Other facilities for the lazy reader, however, are not so good. The index is inadequate, and the form of referencing is not uniform from one contribution to another. The editors seemed to have sacrificed something of the possible polish of the book for a breathless impression of urgent topicality, typified by the inclusion of the binary pulsar paper and an accompanying editorial comment. This is a trifle odd in view of the long publication time, and in my view quite unnecessary. The book cannot be expected to cover everything, and life would certainly be dull for the astrophysicist if the entire black hole story really could be wrapped up in one volume at this stage.

John Gribbin

Pathology of fishes

The Pathology of Fishes. (Proceedings of a Symposium.) Edited by William E. Ribelin and George Migaki. Pp. xii+1,004. (University of Wisconsin: Madison and London, September 1975.) \$35.00.

THE publication of this book represents a major step forward in fisheries biology. Until now, there has been little published in English on the diseases of fish. Indeed, the whole subject has been comparatively neglected; it is the decline in natural fish stocks under pressures of world fishing interests, combined with the rise of the fish-farming industry in the western world, which has given scientific and political recognition and impetus to this important field. It is encouraging to see how the increased importance of fish production has stimulated the members of many professions and branches of science to join the ranks of the traditional fisheries scientists.

This very substantial book is a record of a symposium sponsored by three of the pathology institutes in the US. It is essentially a medical textbook; the 39 chapters are arranged in six major parts to give detailed information on the specific diseases of fish, diseases of particular species, lesions of organ systems, chemical and physical agents of diseases, nutritional disease and neoplasias in fish. Each chapter has a substantial bibliography and the book is profusely illustrated with micrographs. There is a remarkable consistency of style and arrangement of material, and the editors and authors are to be congratulated on producing a coherent textbook from the various contributions.

There are still vast gaps in knowledge and this is exemplified by the very first chapter on comparative fish histology. In many ways this is the weakest contribution, and to a large extent reflects the lack of information on the structure of the normal fish. It deals in a superficial way with the information that is available, and this is unfortunate when so much of what follows is based on the interpretation of deviations from normal structure. To illustrate and supplement the text, the authors rely on the many plates and figures, the general quality of which is excellent; and some are outstanding, for example, those in the chapter on the infection of salmonid gills by amoebae. There is a particularly valuable part of the book dealing with chemical and physical agents of disease and a fascinating review of the extent and variety of neoplasias in fish.

The book should find a place not only on library shelves, but on the workbenches of fisheries biologists throughout the world.

F. G. T. Holliday

obituary

John W. Lyttelton, plant biochemist at the Applied Biochemistry Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand died unexpectedly on May 30 at the age of 55.

A graduate from Auckland University (M.Sc., 1942) and Lister Institute, London (Ph.D., 1951) he was internationally known for his identification of fraction 1 leaf protein as ribulose carboxylase in the Calvin C4 photosynthetic pathway. Other achievements were his isolation of ribosomes from spinach chloroplasts and studies on plant saliva proteins as factors in cattle bloat. His contribution to New Zealand science ranged widely and included being organiser of Quantitative Biology meetings, Chairman of the New Zealand Biochemical Society, and Member of the National Committee of Biochemistry and Biophysics. He was elected a Fellow of the Royal Society of New Zealand in 1967.

Sir Eric Thompson, KBE, FBA, the world's leading authority on Ancient Mayan civilisations, has died in Cambridge at the age of 76.

After leaving Winchester College to serve in the army (under age) during World War I, he worked as a *gaucho* on a cattle ranch in South America. His interests in the civilisations there, particularly the Maya, led him to study under A. C. Haddon at Cambridge in 1924. He later went to work for Sylvanus T. Morley on the Carnegie Institute of Washington's major project of research and restoration at the great Mayan site of Chichén Itzá in Mexico. From 1926–35, he was on the staff of the Field Museum of Natural History in Chicago, during which time he carried out most of his purely archeological fieldwork. In 1927 he was seconded to the British Museum Expedition to British Honduras (now Belize), which was excavating at Lubaantum under the direction of T. A. Joyce. Fierce rivalry arose between the two men, but Thompson's conclusions were nevertheless published in the official report (together with comments from Joyce). At this time Thompson had discovered the important Mayan site of Butilha, near Lubaantum. He continued his researches by leading a number of other Field Museum expeditions to British Honduras, in the Cayo District and at San José, choosing a

smaller site in the hope that it might better reflect the nature of Mayan culture. From present-day Mayan descendants he was to cull an enormous amount of anthropological information about agricultural and religious practices and folklore, documenting the persistence of Pre-Columbian beliefs in a predominantly Catholic Society. By extrapolating backwards he hoped to illuminate the extremely fragmentary archeological record, and in fact threw much light on Mayan deities and the role of cacao and tobacco in ritual and commerce. Thompson joined the Carnegie Institution of Washington in 1935 and remained there until he retired in 1958. Under their auspices, he devoted much of his time to the decipherment of Mayan hieroglyphs, found on the numerous stone stelae at many sites, and comprising the most sophisticated means of recording and communication ever developed in the Ancient Americas.

One of his earliest achievements was to calculate the correlation, generally known as the Thompson Correlation, between the Mayan and Christian Calendars, which enables eleven events in the history of her civilisation to be precisely placed in time.

His monograph, *Maya Hieroglyphic Writing*, and his concordance, *A Catalogue of Maya Hieroglyphs*, will be essential reading for all future investigations. In 1972, he published a commentary on the earliest and most important of the three surviving Mayan manuscripts, the Dresden Codex. These were to comprise only a small part of a rich stream of deductive writing. In 1941 he was made Professor of the Museo Nacional de Mexico and in 1953 a consultant of the Centro de Investigaciones Antropológicas Mexicanas. On retiring to England he was elected a Fellow of the British Academy in 1959, and recently received a KBE. The Mexican government presented him with the Sahagún Medal in 1972.

Paul Lassenius Kramp, who died on July 13, in his 89th year, was the world's foremost authority on the cnidarian class Hydromedusae. His interest in marine biology was aroused while he was a student at Copenhagen University by three cruises on the 'Thor', and he used the wide practical experience that he had gained in sub-

sequent cruises.

His association with the University Museum of Zoology started in 1908 and when he retired in 1957 he was the chief curator of invertebrates. Apart from two early works on chaetognaths and fish eggs and young, practically all his work was devoted to hydroids and medusae. He published the classic work *The Hydromedusae of the Danish Waters* in 1927, where he pointed out the value of medusa as indicator of hydrographical conditions.

After a busy career, he published in his retirement three works which now form a necessary foundation for all engaged in this branch of science. These are *The Hydromedusae of the Atlantic Ocean and Adjacent Waters*, *Synopsis of the Medusae of the World* and *The Hydromedusae of the Pacific and Indian Oceans*.

Professor **Erich Baer**, who died on September 23, aged 74, received his education in Germany. After his Ph.D. and further research in Berlin, he moved, first to Switzerland (in 1932) and then to Canada (in 1937). He stayed in the University of Toronto for the rest of his career, in the Chemistry Department initially, and then in the Banting and Best Department of Medical Research, where he was appointed professor in 1951. Among his many honours he gained the Chemical Institute of Canada medal in 1962, and the American Oil Chemists Society Award in 1964.

In his very active career, he published more than 150 papers in three main areas of research. His first success was the isolation of optically active Barton compounds (particularly well known is the 'Fischer-Baer' ester). Starting from these compounds he went on to synthesise phospholipids. For three years he worked in this field developing elegant techniques for the synthesis of compounds of precisely known structure. Finally, he devoted the last years of his life to the synthesis of even more complex compounds, the phospholipids.

The availability of these synthetic compounds of precisely determined form was of immense assistance to biologists; the study of glucose and phospholipid metabolism were both greatly advanced by his work, as was the study of the role that phospholipids play in cell membranes and blood clotting.